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The Effects of Δ¹-Tetrahydrocannabinol and Other Cannabinoids on Spin-Labeled Liposomes and Their Relationship to Mechanisms of General Anesthesia

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SUMMARY

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The effects of (−)- and (+)-Δ¹-tetrahydrocannabinol (Δ¹-THC), the dimethylheptyl analogue of (−)-Δ¹-THC, 7-hydroxy-Δ¹-THC, cannabinol, and cannabidiol on the molecular mobility of the hydrocarbon phase of lecithin/cholesterol ultrasonically dispersed vesicles (liposomes) were investigated using a nitroxide-labeled dipalmitoyllecithin as a molecular probe. At low concentrations (−)-Δ¹-THC fluidized the lipid bilayer; at Δ¹-THC to lecithin ratios greater than 0.05:1 the effect leveled off and the change in the order parameter remained constant and independent of the membrane concentration. The maximum increase in bilayer fluidity produced by Δ¹-THC was considerably less than that produced by general anesthetics at membrane concentrations corresponding to those producing anesthesia *in vivo*. (+)-Δ¹-THC (the "unnatural" optical isomer of Δ¹-THC) was less effective than (−)-Δ¹-THC, whereas the dimethylheptyl analogue and 7-hydroxy-Δ¹-THC were more effective. Hence ability, at low concentrations, to disorder liposome bilayers correlates well with psychoactive potency. Cannabinol and cannabidiol decreased the fluidity of the liposome bilayer. Octanol, a potent general anesthetic, fluidized lipid bilayers; hexadecanol and tetradecane (which do not produce anesthesia *in vivo*) were less effective than Δ¹-THC in fluidizing liposomes and, at high doses, produced a cannabis-like cataleptic state in mice. It is suggested that the psychoactive cannabinoids may be classified as "partial anesthetics," producing a perturbation of the membrane structure qualitatively similar to that produced by subanesthetic doses of general anesthetics, but which, because of their limited solubility in the lipid phase of cell membranes, are unable to produce the degree of membrane disorder corresponding to clinical anesthesia.

INTRODUCTION

Unlike most centrally active compounds, the cannabinoids (e.g., Δ¹-tetrahydrocannabinol, I) possess no basic nitrogen atom, and their general lack of chemical similarity to any of the other hallucinogenic compounds, together with their lack of cross-tolerance, suggests that the cannabinoids have a fundamentally different mode of action at the molecular level from such compounds as lysergic acid diethylam-

[1] This work was carried out during the tenure of a Medical Research Council training award.

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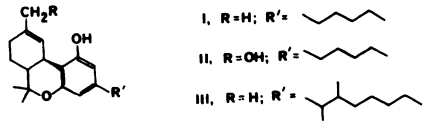
ide, psilocybine, or mescaline. The lack of basic groups, combined with very high lipid solubility, has suggested analogy with the general anesthetics and, in particular, with the recently developed steroid anesthetics (1). General anesthetic properties are exhibited by a wide range of low molecular weight volatile compounds, and such activity is usually explained in terms of an ability to expand and fluidize the hydrophobic components of nerve cell membranes (2). Variations in potency are correlated with physical properties such as lipid solubility, and anesthetic potency is not considered to be dependent on chemical structure. On the other hand, extensive studies of the structure-action relationships of compounds related to Δ^1-THC[3] showed that minor variations in chemical structure could produce marked variations in potency when assayed for a range of central nervous system activities in animals (3) and in man (4). Later studies with a more limited range of tritium-labeled derivatives (5–8), in which behavioral effects were correlated with gross brain concentrations, both of the parent compound and of its metabolites, showed that variations in one such effect could not be accounted for by failure to reach the central nervous system or by major differences in metabolism. These variations in potency within the cannabinoids appeared to be intrinsic to their chemical structures, and in such cases an interaction with a specific receptor protein is usually proposed, variations in potency being ascribed to the closeness of fit of the drug molecule to the matrix of side chain groups of the receptor.

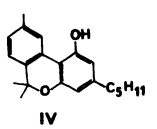
The nerve cell membrane is a complex lipid-protein structure, consisting of a bimolecular layer of phospholipid and cholesterol which either is overlaid by a layer of protein (the classicial Davson-Danielli model) or is penetrated by clusters of globular protein molecules [the mosaic model (9)]. With techniques at present available it is not possible to distinguish between the two possible sites of drug action—protein or the lipid bilayer—by experiments on intact cell membranes, but this difficulty can, in part, be circumvented by studies of model systems. Ultrasonically dispersed suspensions of lecithin and cholesterol have been shown to consist of spherical vesicles of varying diameters (liposomes) with the lipid molecules stacked together in bimolecular layers to produce a structure very similar to the lipid phase of cell membranes (10). Hubbell and McConnell (11) have described the synthesis of a nitroxide-labeled dipalmitoyllecithin and its use as a probe to determine the degree of disorder of the hydrocarbon chains of the lipid bilayer by measurement of selected hyperfine splitting parameters of the electron spin resonance signal of the nitroxide radical. Using this technique, it has been shown (12) that within a series of steroid derivatives ability to fluidize the protein-free liposome bilayer correlated well with anesthetic potency, making it unnecessary to postulate the existence of a protein receptor substance, and supporting the hypothesis that the lipid phase of nerve cell membranes is the site of action of all general anesthetics. As an extention of these studies, the effects of a group of cannabinoids on spin-labeled liposomes have now been investigated.

[3] The abbreviation used is: Δ^1-THC, Δ^1-tetrahydrocannabinol.

MATERIALS AND METHODS

(−)Δ^1-THC was isolated from crude cannabis (13); the syntheses of the other cannabinoids, namely, (+)-Δ^1-THC (7), 7-hydroxy-Δ^1-THC (II) (6), and the 1,2-dimethylheptyl analogue of Δ^1-THC (III) (8), have been described. The sample of (+)-Δ^1-THC contained not more than 3% of its enantiomorph. Cannabinol (IV) was obtained from Makor Chemicals, and the cannabidiol (V) used was a generous gift from





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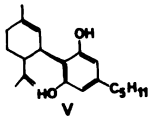
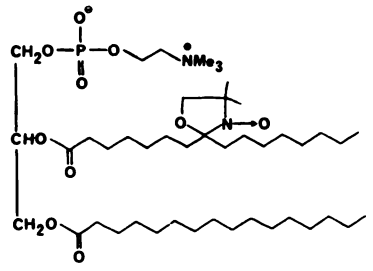


V

Professor R. Mechoulam. Egg lecithin (grade I) and synthetic L-3-α-dipalmitoyllecithin were obtained from Koch-Light Laboratories; octanol, hexadecanol, tetradecane, and cholesterol were obtained from British Drug Houses. Spin-labeled dipalmitoyllecithin (VI) was synthesised as described by Hubbell and McConnell (11).



VI

Electron spin resonance techniques. All ESR spectra were recorded on a JEOL PES-1 ESR spectrometer at a temperature of 22° ± 1° using X band radiation at 9.45 ± 0.05 GHz. The detecter modulation width was kept low to avoid artificial broadening of the signal. Calibration was done with diphenylpicrylhydrazyl and Mn^{2+}.

The procedure for preparing lecithin/cholesterol liposomes containing varying amounts of drug has been described in detail (12). Briefly, a chloroform solution of the appropriate quantities of cannabinoid, lecithin, and cholesterol was evaporated under nitrogen to produce an intimate mixture, which was then dispersed in 0.9% NaCl solution using an MSE 100-W ultrasonic disintegrator. The molar ratio of lecithin to cholesterol was kept constant at 1:0.9, a ratio chosen to produce a fairly rigid membrane and to enable comparisons with other observations (12, 14). The molar ratio of lecithin to cannabinoid was varied over the range 16:1–2:1.

Calculation of "order parameter." The nitroxide group of the modified dipalmitoyllecithin (VI) has an uncoupled electron, and the spin resonance signal of this electron is dependent on the mobility of that part of the molecule to which it is attached and hence, in a condensed phase, on the constraint imposed by adjacent molecules. Following the treatment due to Hubbell and McConnell (11), the geometry of the nitroxide group attached to an aliphatic chain is such that measurements of line width give a measure of torsional freedom parallel to, and at right angles to, the hydrocarbon chain. These two parameters are combined in a composite "order parameter," S, which gives a measure of the degree of local molecular order in the immediate environment of the spin label, increasing as this environment becomes more closely packed, decreasing as the lipid phase is disordered and expanded. The procedure for calculating S was that described in a previous paper (12).

Bioassays. For administration to mice (male albino, 23–27 g, Tuck strain No. 1) cannabinoids, octanol, hexadecanol, and tetradecane were dispersed with Tween 80 in 0.9% NaCl. The amount of Tween 80 injected was kept constant (20 mg/kg), and the same dose of Tween 80 in NaCl was used in controls. Injections were made into a lateral tail vein in volumes of 0.2 ml/25 g. Immobility indices were measured using the ring test (15).

RESULTS

It was found that (−)- and (+)-Δ^1-THC, 7-hydroxy-Δ^1-THC, and the dimethylheptyl analogue of Δ^1-THC all caused disordering of the liposome bilayer (Table 1). As was found with anesthetic steroids (12), the magnitude of the effect was the same with both natural and synthetic lecithin, and Table 1 combines results from both types of liposome. In order to facilitate comparisons between molecules of different sizes, liposome concentrations are also expressed in terms of the partial molar volume (expressed in cubic centimeters per mole of lecithin) of the liposome occupied by the drug (16), per mole of lecithin and per 100 ml of lecithin/cholesterol.

The effect of (−)-Δ^1-THC on the degree of molecular order of the lecithin hydrocarbon chains was biphasic. At low concentrations the lipid layer was fluidized, the extent of the disorder produced increasing with concentration over a limited range. However, in contrast to the effect produced by the potent steroid anesthetics, the perturbation of the liposome produced by Δ^1-THC was limited in magnitude and the

TABLE 1

Effects of various cannabinoids, alkanols, and alkanes on disordering of dipalmitoyl and egg lecithin/cholesterol liposomes, expressed as changes in order parameter, S

The concentration of drug in the membrane is expressed as cubic centimeters per mole of lecithin and as cubic centimeters per 100 ml of lecithin/cholesterol (lecithin, 475 cm^3/mole; cholesterol, 253 cm^3/mole). Each value is the mean ± standard deviation of four to six readings. Significant differences between the effects of the same doses of the enantiomorphs of Δ^1-THC were obtained by Student's *t*-test.

Drug (molal volume)	Concentration	Partial volume	Drug volume	$-\Delta S$
	mmoles/mole lecithin	*cm^3/mole lecithin × 10*	*cm^3/100 ml membrane lipid*	
(−)-Δ^1-THC (203 cm^3)	6.3	13	1.85	0.004 ± 0.007
	12.6	26	3.7	0.009 ± 0.006
	25	51	7.25	0.015 ± 0.006
	50	102	14.5	0.019 ± 0.008
	100	203	28.9	0.020 ± 0.004[a]
(+)-Δ^1-THC (203 cm^3)	200	406	57.7	0.024 ± 0.003[a]
	25	51	7.25	0.002 ± 0.006
	50	102	14.5	0.006 ± 0.008
Dimethylheptyl analogue (244 cm^3)	100	203	28.9	0.009 ± 0.008[a]
	200	406	57.7	0.011 ± 0.008[a]
	1	2	0.28	0.012 ± 0.007
	2	5	0.71	0.024 ± 0.008
	4	10	1.42	0.032 ± 0.010
7-Hydroxy-Δ^1-THC (207 cm^3)	8	20	2.84	0.034 ± 0.008
	32	78	11.1	0.055 ± 0.013
	64	156	22.2	0.061 ± 0.013
Cannabinol (201 cm^3)	10	21	2.98	0.020 ± 0.014
	20	41	5.83	0.037 ± 0.011
	40	83	11.8	0.045 ± 0.008
Cannabidiol (206 cm^3)	80	166	23.6	0.049 ± 0.016
	12.5	25	3.55	−0.002 ± 0.006
	25	50	7.11	−0.019 ± 0.006
Octanol (93 cm^3)	50	100	14.22	−0.027 ± 0.008
	100	201	28.6	−0.043 ± 0.013
Hexadecanol (175 cm^3)	12.5	26	3.7	−0.002 ± 0.006
	25	51	7.25	−0.009 ± 0.006
	50	103	14.64	−0.014 ± 0.006
Tetradecane (150 cm^3)	100	206	29.30	−0.034 ± 0.009
	250	232	33.0	0.045 ± 0.008
	500	464	66.0	0.092 ± 0.009
	100	175	24.9	0.007 ± 0.007
	200	350	49.8	0.009 ± 0.006
	400	700	99.6	0.012 ± 0.008
	100	150	21.35	0.008 ± 0.006
	200	300	42.70	0.012 ± 0.008
	400	600	85.40	0.016 ± 0.006

[a] $p < 0.05$.

effect leveled off as the amount of Δ^1-THC in the liposome was increased (Fig. 1). (+)-Δ^1-THC, the enantiomer of the naturally occurring compound, was less effective than (−)-Δ^1-THC in disordering the liposome, in line with its reduced biological activity (7). The effects produced by both compounds were small, and at low concentrations the difference between the effects produced was not statistically significant;

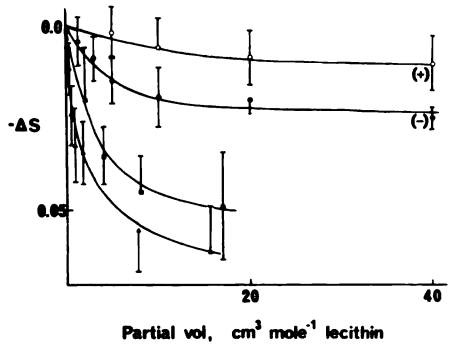


FIG. 1. *Changes in order parameter of lecithin/cholesterol liposomes plotted against partial drug volume in bilayer*

●, (−)-Δ^1-THC; ○, (+)-Δ^1-THC; ■, 7-hydroxy-Δ^1-THC; ▲, dimethylheptyl analogue of (−)-Δ^1-THC. Each point is the mean ± standard deviation of four to six readings.

at higher concentrations, when the effects of both compounds had leveled off, the difference between the two enantiomers was statistically significant ($p < 0.05$), and it would appear that the liposome bilayer is sufficiently chiral to differentiate between optical isomers. The major metabolite of Δ^1-THC, 7-hydroxy-Δ^1-THC (II), and the dimethylheptyl analogue of Δ^1-THC (III) were both more effective in disordering the liposome than Δ^1-THC, and again the extent of the perturbation appeared to level off with increasing membrane concentration. Unfortunately, in the case of these two latter compounds, insufficient material was available to examine their effects over a wider concentration range.

In contrast to the fluidizing effect produced by the psychoactive cannabinoids, cannabinol and cannabidiol both decreased the fluidity of the liposome (Fig. 2). In both cases the increase in the order parameter produced by lecithin to cannabinoid molar ratios of 20:1 or less was significantly greater than that recorded for liposomes in the absence of added drug ($p < 0.05$).

The leveling off of the perturbation of the liposome bilayer as the amount of Δ^1-THC added is increased might be due to the limited solubility of the latter in the phospholipids. In many homologous series of compounds the geometrical increase in narcotic potency observed as the series is ascended is terminated by the limited solubility of the higher members of the series in the relevant biophase (17). It was therefore of interest to examine the effects on spin-labeled liposomes of other compounds above and below the narcotic cutoff point. The cutoff point in any particular series of compounds depends on the bioassay procedure used but usually occurs at about decane for *n*-alkanes and dodecanol for 1-alkanols.

Figure 3 shows the effects of octanol, hexadecanol, and the linear hydrocarbon tetradecane. Octanol is an effective general anesthetic when administered to animals intravenously, and it has previously been shown (12) to be as effective as butobarbitone in disordering spin-labeled liposomes. These observations are reproduced in Fig. 3. It can be seen that the effects produced by hexadecanol and tetradecane are very much smaller. The decrease in order parameter is so small that it is not possible to determine whether these two compounds also produce a biphasic effect or whether the effect is increasing linearly with concentration, but with a gradient very close to zero. In any event it is clear that even at very high liposome levels neither compound would produce a perturbation corresponding to that produced by potent general anesthetics at anesthetic doses (12).

Intravenous doses of hexadecanol (100 mg/kg) or tetradecane (70 mg/kg) dis-

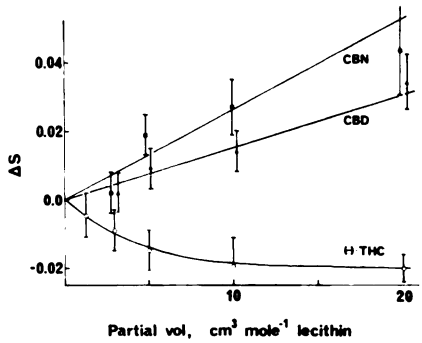


FIG. 2. *Changes in order parameter of lecithin/cholesterol liposomes plotted against partial drug volume in bilayer*

■, cannabinol (CBN); ▲, cannabidiol (CBD); ○, Δ^1-THC. Each point is the mean ± standard deviation of four to six readings.

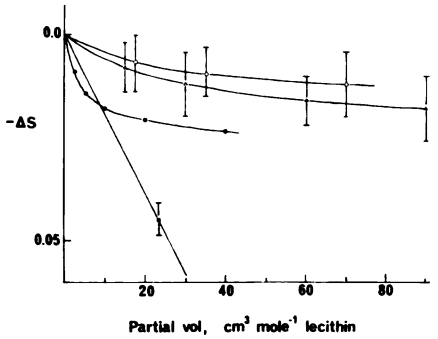


FIG 3. *Changes in order parameter of lecithin/cholesterol liposomes plotted against partial drug volume in bilayer*

■, octanol; ○, hexadecanol; △, tetradecane; ●, Δ^1-THC. Each point is the mean ± standard deviation of four to six readings.

persed with Tween 80 (20 mg/kg) in NaCl produced a "cataleptic" state, as measured by the ring immobility test (15), indistinguishable from that produced by Δ^1-THC (2 mg/kg).

DISCUSSION

These experiments were designed to test the hypothesis that Δ^1-THC and its psychoactive analogues exert their primary pharmacological action in a manner analogous to the general anesthetics, that is, through an effect on the lipid phase of nerve cell membranes. The two most obvious difficulties with such a hypothesis are (a) that the central nervous system activities of the cannabinoids show quite a marked dependence of activity on chemical structure, and chemical specificity is not one of the generally recognized characteristics of general anesthetics, and (b) that Δ^1-THC and its psychoactive congeners do not produce anesthesia in experimental animals, even when injected in large doses.

Previous work (12) has demonstrated structure-dependent effects of a range of steroids on protein-free spin-labeled liposomes, ability to fluidize the liposome bilayer correlating well with anesthetic potency. Assuming that the anesthetic effect produced by these steroids is a consequence of this fluidization of the lipid phase of the nerve cell membrane, this effect would account for the observed structure-action relationships within the steroid series of anesthetics, making it unnecessary to postulate the existence of a specific receptor substance. In the same way the psychoactive cannabinoids related to Δ^1-THC, at low concentrations, produce a qualitatively similar increase in the disorder of spin-labeled liposomes, the order of effectiveness in decreasing the order parameter within the group of compounds studied correlating well with their ability to produce central nervous system effects. The effect of the cannabinoids on cholesterol/lecithin bilayers is therefore qualitatively the same as that of a subanesthetic dose of an anesthetic, and we may suppose that the effect of the cannabinoids on neuronal membranes is the same.

The maximum disordering effect produced by Δ^1-THC corresponded to a ΔS value of 0.024, at a membrane concentration of 200 mmoles/mole of lecithin. This is a relatively weak effect when compared to that produced by potent anesthetics. Anesthesia is said to occur at a membrane drug partial volume of about 3 cm^3/kg of dry membrane (2), equivalent to about 20 cm^3/mole of lecithin. At such membrane concentrations potent general anesthetics produce a perturbation of lipid bilayers corresponding to a ΔS value of about 0.06 (12). Hence the disordering of the liposome bilayer produced by Δ^1-THC falls far short of that corresponding to an anesthetic effect *in vivo*, and Δ^1-THC would not be expected to produce anesthesia, by itself, even if given in very large doses.

This self-limiting effect could be due to the limited solubility of Δ^1-THC in the core of the lecithin/cholesterol bilayer: at higher liposome loadings Δ^1-THC presumably absorbs to the liposome in some alternative configuration that does not lead to further perturbation of the hydrocarbon core. Such an explanation is analogous to that relating the abrupt loss of anesthetic potency as a homologous series is ascended to a limited solubility in the biophase. As Δ^1-THC is capable of causing marked behavioral changes without being able to produce anesthesia, it was possible that hexadecanol and tetradecane could produce behavioral effects similar to those produced by Δ^1-THC, and this proved to be the

case. Thus it would appear that the cannabinoids, like the long-chain alkanols and alkanes above the "cutoff" point in their respective homologous series, can be described as partial anesthetics, their effects qualitatively resembling those of subanesthetic doses of anesthetics, but with the effect being self-limiting before anesthesia is produced.

The enantiomorphic form of Δ^1-THC, (+)-Δ^1-THC, was found to be less effective than the naturally occurring compound in fluidizing liposomes, which parallels its lesser central nervous system activity (7). The magnitude of the effect is small but, at high concentrations, is statistically significant. (+)-Δ^1-THC is at least 13–20 times less active than its isomer, and its effect on liposomes is comparable to that produced by hexadecanol. The latter is roughly 50 times less active than Δ^1-THC, and so the physical and biological effects of these compounds correlate reasonably well within the wide limits of error of the measurements.

The demonstration of optical specificity in the interaction of a lecithin/cholesterol bilayer with an adsorbed molecule is of some theoretical interest. It has been suggested that general anesthetics would not be expected to exhibit stereospecificity (18), and in one of the rare cases when this proposition has been tested with a volatile anesthetic it was found that the two optical isomers of halothane were equiactive in their ability to depress synaptic transmission in an isolated ganglion preparation and to disorder spin-labeled liposomes (14). However, despite some assertions to the contrary (18), enantiomeric pairs of barbiturates, with an asymmetrical center in the carbon side chain, have been shown to differ in their hypnotic potency, without any difference in their ability to penetrate to the central nervous system (19). Cholesterol is an important constituent of cell membranes and is a component of the liposomes used in the work reported here; it is optically active and it would be expected to impose some degree of chirality on the hydrocarbon core of the lipid bilayer. This anisotropy would probably be more significant in the case of large molecules such as the cannabinoids, which are approximately the same size as cholesterol and would occupy the full depth of one molecular layer of the bilayer structure, rather than for much smaller molecules such as halothane, which is a halogenated ethane derivative. The demonstration of stereoselectivity by a lecithin/cholesterol bilayer structure is further support for the proposition that it is the lipid phase of the neuronal membrane that is the primary site of action of the cannabinoids, and that it is unnecessary to invoke the existence of a specific cannabinoid receptor.

Cannabinol and cannabidiol increase the molecular order of the liposome bilayer, making the structure more rigid. As this is the opposite effect to that produced by the psychoactive cannabinoids, it might be anticipated that these two substances would antagonize the effects of Δ^1-THC. Cannabinol and cannabidiol do not produce any very marked behavioral changes when administered to animals by themselves (20), and so they have not attracted the same degree of interest as the other psychoactive cannabinoids. However, the possibility of interaction between the various constituents of crude cannabis has been suggested by many workers, and Carlini *et al.* have demonstrated that cannabidiol can block the excitatory effects and potentiate the depressant effects of Δ^1-THC in rats (21) and in man (22). Similarly, Kranz, Berger, and Welch (23) found that cannabinol antagonizes the pentobarbitone-potentiating properties of Δ^1-THC on sleeping time in rats, and Chester, Jackson, and Starmer (24) have confirmed this observation in mice. The effect of cannabinol and cannabidiol is to make the lipid layer more rigid and orderly, but it is not yet clear what the consequences of such a change would be on nerve cell excitability. The fact that cannabidiol and cannabinol have the opposite effect to the other psychoactive cannabinoids on lipid bilayers does, however, mean that the cannabinoids cannot all be treated as homologous with one another, and comparisons between the biological effects of cannabinol, say, on the one hand, and Δ^1-THC, on the other hand, must be made with care.

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