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Effects of the Cannabinoids on Physical Properties of Brain Membranes and Phospholipid Vesicles: Fluorescence Studies[1]

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ABSTRACT

The effects of four cannabinoids on the physical properties of brain synaptic plasma membranes (SPM), lipid extracts of SPM and phospholipid vesicles were evaluated using fluorescence probes. *In vitro*, the psychoactive cannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC) and 11-hydroxyl-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC) at concentrations of 1 and 3 μM decreased polarization of the fluorescence emission of 1,6-diphenyl-1,3,5-hexatriene (DPH) in SPM. At the same concentrations, cannabidiol (CBD) and cannabinol, cannabinoids devoid of marijuana-like psychoactivity, had no effect on DPH polarization. The effects of 11-OH-Δ^9-THC and CBD on vesicles made from lipids extracted from SPM were identical to their effects on intact SPM. These changes in DPH polarization were not due to changes in fluorescence lifetime and indicate that, at low concentrations, the psychoactive cannabinoids increase the rotational mobility of DPH in the membrane core. In contrast, in SPM-extracted lipids, both 11-OH-Δ^9-THC and CBD decreased the mobility of stearic acid with an anthroyloxy label at both the second (2-AS) and twelfth (12-AS) carbon atoms. Studies of DPH polarization in various phosphatidylcholines (PC) demonstrated that the actions of the cannabinoids were dependent on initial bilayer fluidity. 11-OH-Δ^9-THC was less effective at decreasing polarization of trimethylammonium DPH (TMA-DPH), a probe of the bilayer surface, than of DPH whereas CBD affected mobility of the two probes equally. Neither CBD nor 11-OH-Δ^9-THC altered DPH mobility in phosphatidylethanolamine, phosphatidylserine vesicles. These findings indicate that the psychoactive cannabinoids increase fluidity in the hydrophobic core of brain membranes and support a membrane perturbant hypothesis of the mechanism of Δ^9-THC action.

The molecular mechanisms for the psychotropic action of Δ^9-THC, the principal active component of marijuana, are not well understood. It has been suggested that Δ^9-THC exerts some of its effects through interactions with lipid constituents of nerve cell membranes (Lawrence and Gill, 1975; Paton, 1975; Roth and Williams, 1979). Circumstantial support for this hypothesis includes the wide variety of membrane-associated enzymes (Banerjee *et al.*, 1975; Bloom *et al.*, 1978); neurotransmitter receptors (Hillard and Bloom, 1982; Bloom *et al.*, 1982) and uptake processes (Lindamood and Colasanti, 1980; Poddar and Dewey, 1980) that are affected by the cannabinoids. The functioning of many membrane-associated proteins can be affected by changes in membrane physical properties (Sandermann, 1978; Loh and Law, 1980); therefore, it is possible that cannabinoid-induced changes in membrane proteins occur secondarily to changes in membrane lipid ordering or viscosity.

In direct support of this membrane perturbation hypothesis of cannabinoid action, several investigators have demonstrated cannabinoid-induced changes in the "fluidity" of phospholipid vesicles. Lawrence and Gill (1975) reported that Δ^9-THC and 11-OH-Δ^9-THC both increased the mobility of nitroxide-labeled PC in egg yolk PC/CHOL vesicles whereas CBD and CBN decreased probe mobility. Proton NMR studies (Tamir and Lichtenberg, 1983) have confirmed the findings of Lawrence and Gill (1975) and suggest that the presence of CHOL is essential for the bilayer perturbant activity of the cannabinoids. Recently, the interactions of Δ^9-THC with PC bilayers were studied using differential scanning calorimetry (Bruggeman and Melchior, 1983). In these studies, Δ^9-THC was found to form complexes with PC bilayers and to interact preferentially with the more fluid areas of the bilayers.

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ABBREVIATIONS: Δ^9-THC, Δ^9-tetrahydrocannabinol; 11-OH-Δ^9-THC, 11-hydroxyl-Δ^9-THC; PC, phosphatidylcholine; CHOL, cholesterol; CBD, cannabidiol; CBN, cannabinol; NMR, nuclear magnetic resonance; SPM, synaptic plasma membranes; PBS, phosphate-buffered saline; DPPE, dipalmitoyl phosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-(4-trimethylammonium phenyl)-DPH; 2-AS, 2-(9-anthroyloxy) stearic acid; THF, tetrahydrofuran; DMSO, dimethylsulfoxide; DPOPC, dipalmitoleoyl PC; DMPC, dimyristoyl PC; DPPC, dipalmitoyl PC; T_m, transition temperature; PE, phosphatidylethanolamine; PS, phosphatidylserine; ESR, electron spin resonance.

From these studies, it is apparent that Δ^9-THC can alter the physical properties of phospholipid vesicles. However, several important questions remain to be answered. 1) Do the cannabinoids also perturb lipids in biological membranes? 2) Do the membrane perturbant potencies of the cannabinoids correlate with their psychoactive potencies? 3) Do the cannabinoids have different effects on different membranes? In this report, we describe studies of the effects of several cannabinoids on the polarization and lifetime of fluorescence of probe molecules in SPM. In addition, we compare the effects of the cannabinoids on acyl chain ordering in lipid extracts of SPM and in vesicles formed from a series of phospholipids.

Methods

Preparation of the SPM. SPM were prepared from the whole brains of male, Sprague Dawley rats or DBA/2N mice (Simonsen Labs, Gilroy, CA) by Ficoll and sucrose density centrifugation as described previously (Harris and Schroeder, 1982). Membranes were suspended in PBS (8 g/l of NaCl, 0.2 g/l of KCl, 0.2 g/l of KH_2PO_4, 1.45 g/l of $Na_2HPO_4 \cdot 7H_2O$ and 0.48 g/l of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4) at a concentration of 1 to 3 mg of protein per ml and were stored at −20°C until use. In polarization studies, SPM were diluted to 50 μg of protein per ml with PBS and, in lifetime studies, SPM were diluted to 100 μg of protein per ml. Protein was determined using the method of Lowry *et al.* (1951).

Lipid extraction. To prepare a "total" lipid extract, mouse brain SPM were prepared using a modification (Harris and Schroeder, 1982) of the method described by Cotman and Matthews (1971). The pellet was suspended in methanol and mixed with an equal volume of chloroform under nitrogen. After vortexing, the solution was kept at −20°C for 1 to 2 hr, then centrifuged. The resulting pellet was extracted with chloroform-methanol (2:1) and centrifuged. The two supernatants were combined to give a total lipid extract which was stored under argon at −20°C. This extraction procedure removes all membrane lipids and less than 3% of the membrane protein (Harris *et al.*, 1984). Vesicles were prepared from the extracted SPM lipids as described previously (Harris and Schroeder, 1981).

Vesicle preparation. Lipids were obtained from Avanti Polar Lipids (Birmingham, AL) except for DPPE, egg yolk PC and CHOL which were obtained from Sigma Chemical Company (St. Louis, MO). No impurities in the lipids were detected by thin-layer chromatography (silica gel H; $CHCl_3$-CH_3OH-NH_4OH; 14:7:1) and they were used without purification. The lipids were dissolved in chloroform (2 nmol/μl) and were stored under argon at −20°C. To make the vesicles, aliquots of lipid stock were placed into glass culture tubes and were dryed under a stream of nitrogen. PBS was added to each tube (total lipid concentration was 90 nmol/ml) and the tubes were vortexed then sonicated for 30 sec in a bath sonicator. This procedure gives large, multilamellar vesicles.

Fluorescence measurements. An HH-1 T-format polarization spectrofluorometer (H and L Instruments, Burlingame, CA) with fixed excitation and emission polarization filters was used to measure fluorescence intensity parallel (I_{11}) and perpendicular (I_1) to the polarization plane of the exciting light (Harris and Schroeder, 1982). Polarization of fluorescence $[(I_{11}-I_1)/(I_{11}+I_1)]$ was calculated by an on-line microprocessor. DPH, TMA-DPH, 2-AS and 12-AS were obtained from either Molecular Probes, Inc. (Junction City, OR) or Aldrich Chemical Company (Milwaukee, WI). For DPH and TMA-DPH, the excitation wavelength was 362 nm and for 2-AS and 12-AS the excitation wavelength was 363 nm. A 03FCG001 filter (band pass of 310 to 390 nm; Melles Griot, Irvine, CA) was used in the excitation beam and kV 389 filters (cutoff below 389 nm; Schott Optical, Duryea, PA) were used for the emitted light.

Fluorescence lifetime measurements were made using a PRA System 3000 Fluorescence Lifetime Instrument (London, Ontario, Canada) using a flash lamp excitation source. Pulse duration was 2 nsec and had a frequency of 30 kHz. A Tracor Northern TN-7200 multi-channel analyzer (Middleton, WI) was used for signal acquisition and digitization. Data were deconvoluted and lifetimes were calculated using the Deconvolution Decay Version 3.0 computer program by PRA.

In all studies, cuvette temperature was controlled to within ± 0.1°C and was monitored continuously by a thermocouple inserted into the cuvette to a level just above the light beam. Temperature was 35°C unless noted otherwise.

Probes were dissolved in THF (DPH); THF-water (1:1) (TMA-DPH) or methanol (2-AS and 12-AS). In the polarization studies, probes were added to SPM or vesicle suspension in a volume of 0.3 to 0.5 μl/ml suspension to give a probe concentration of 0.04 to 0.8 μg/ml (DPH, TMA-DPH and 12-AS) or 0.3 μg/μl (2-AS). In the lifetime studies, DPH was added in a volume of 1 μl/ml of suspension to give a probe concentration of 10 μg/ml. The probes were incorporated into SPM, extracted lipid vesicles and phospholipid vesicles at 35°C for 15 min with frequent vortexing. The suspensions were transferred to quartz cuvettes for fluorescence measurements.

Drugs. Δ^9-THC, 11-OH-Δ^9-THC, CBN and CBD were all generously supplied by National Institute of Drug Abuse (Rockville, MD). The cannabinoids were added to the SPM or vesicle suspensions using DMSO as a vehicle. No more than 10 μl of DMSO per ml of suspension was added and this concentration of DMSO had no effect on fluorescence polarization in any preparation. Δ^8-[^{14}C]THC was obtained from Research Products International Corp. (Mount Prospect, IL) and was administered using an Emulphor (GAF, New York, NY)-ethanol-saline vehicle (Craddock *et al.*, 1973).

Data analysis. The effects of drug addition on fluorescence polarization in SPM and extracted SPM lipid vesicles were evaluated overall using two-way analysis of variance. Individual comparisons between control and each drug concentration were made using Duncan's Multiple range test. Drug effects on DPH fluorescence lifetime were evaluated using one-way analysis of variance followed by *t* tests.

Results

The effects of the four cannabinoids, Δ^9-THC, 11-OH-Δ^9-THC, CBD and CBN on polarization of DPH fluorescence in rat brain SPM were determined (fig. 1). At a concentration of 1 μM, Δ^9-THC produced a significant decrease in DPH fluorescence polarization. At 3 μM, both Δ^9-THC and 11-OH-Δ^9-THC significantly decreased DPH fluorescence polarization. Neither CBN nor CBD had any significant effect on DPH polarization at the same concentrations. As discussed below, these results indicate that, at low concentrations, Δ^9-THC and 11-OH-Δ^9-THC increased the mobility of DPH within the membrane. At higher concentrations, 11-OH-Δ^9-THC continued to produce a concentration-related decrease in DPH fluorescence polarization. However, 30 μM concentrations of Δ^9-THC, and CBD and CBN all significantly increased DPH fluorescence polarization, indicating that these drugs decrease DPH mobility at high concentrations.

To assess the physiological relevance of the *in vitro* concentrations of the cannabinoids used in these studies, the cerebral cortical concentration of Δ^8-[^{14}C]THC was determined after *in vivo* administration. Δ^8-[^{14}C]THC was administered to mice by i.v. injection at a dose of 3.8 mg/kg, a moderately hypothermic dose. Assuming that the specific gravity of cerebral cortical tissue is 1.0 g/ml, the overall concentration of Δ^8-[^{14}C]THC and metabolites in the cerebral cortex was 3.17 ± 0.09 μM at 1 hr after injection. Therefore, the cannabinoid concentrations used in these studies are in a reasonable range.

The role of membrane proteins in the effects of the cannabinoids on DPH mobility in SPM was assessed in total lipid extracts of mouse brain SPM. Mouse brain intact SPM respond

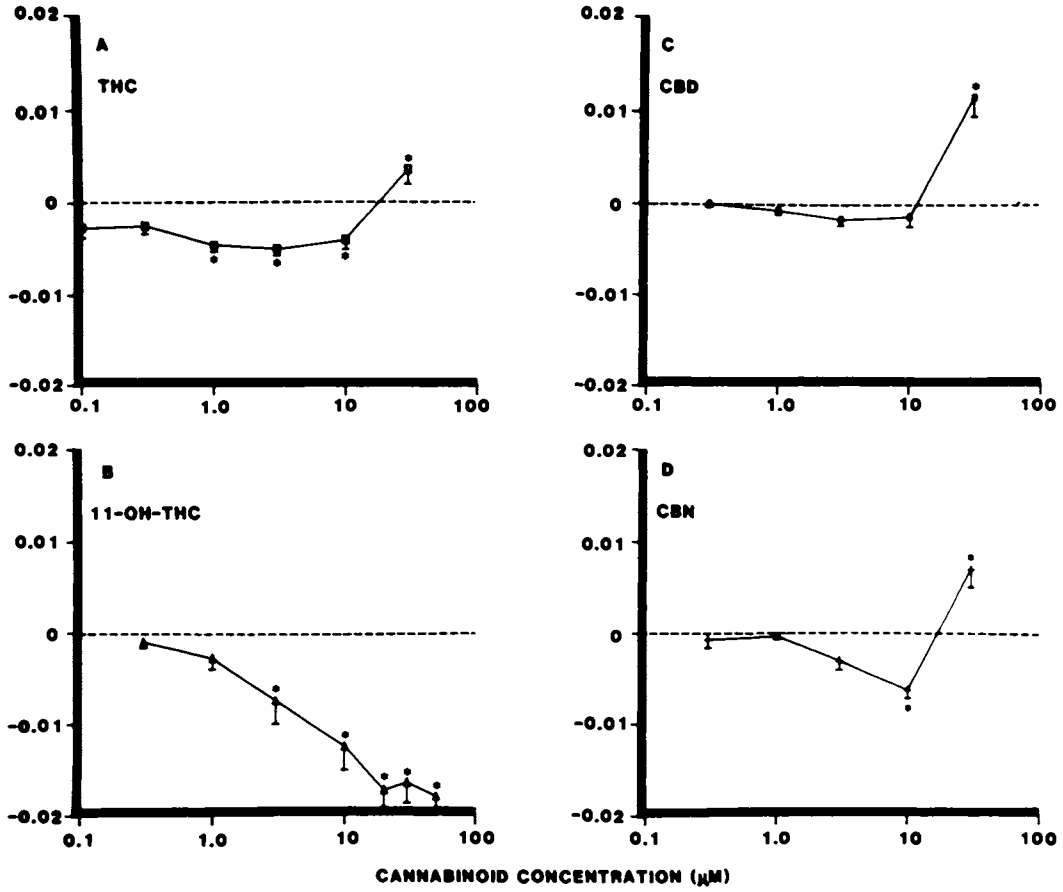

Fig. 1. Effects of cannabinoids on fluorescence polarization of DPH in rat brain SPM. Cannabinoids were dissolved in DMSO and added to SPM suspensions at the concentrations indicated. Fluorescence polarization of DPH was determined at 35°C. Vertical lines represent S.E.M. For Δ^9-THC (A), n = 5; for 11-OH-Δ^9-THC (B); CBD (C), n = 4; and for CBN (D) n = 3. The mean predrug polarization was 0.261 ± .001. * $P < .05$ compared to pretreatment polarization.

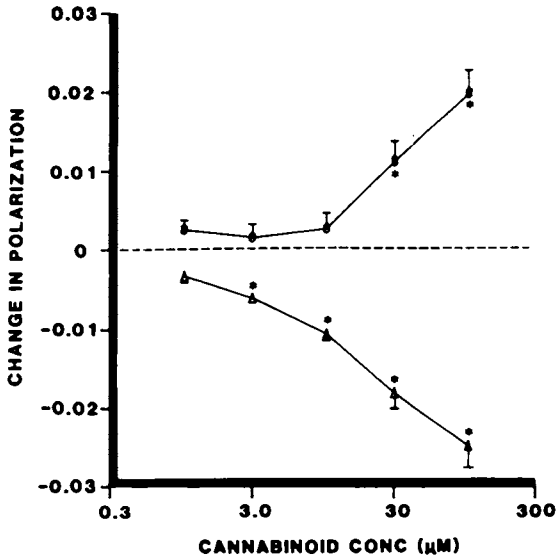

Fig. 2. Effects of cannabinoids on fluorescence polarization of DPH in total lipid extract of mouse brain SPM. Cannabinoids were dissolved in DMSO and added to vesicles prepared from a total lipid extract of mouse brain SPM. Fluorescence polarization of DPH was determined at 35°C. For 11-OH-Δ^9-THC (Δ), n = 6 and for CBD (O), n = 5. The mean predrug polarization was 0.252 ± 0.004. * $P < .05$ compared to pretreatment polarization. CONC, concentration.

to the cannabinoids in a manner similar to rat brain SPM (data not shown). In vesicles formed from extracted lipids, 11-OH-Δ^9-THC significantly decreased fluorescence polarization of DPH at concentrations of 3 μM and greater (fig. 2). In contrast, CBD significantly increased DPH polarization at concentrations of 30 and 100 μM. These effects are similar to the effects of those two cannabinoids on intact rat (and mouse) SPM. Thus, membrane proteins are not required for the effects of these drugs on DPH polarization.

DPH localizes preferentially near the center of the membrane bilayer in the methyl terminal region of the acyl chains (Lentz *et al.*, 1976). To compare the effects of the cannabinoids on the membrane core with their effects on different levels of the membrane bilayer, two anthroyloxy fatty acids, 2-AS and 12-AS, were used to probe the areas of the glycerol backbone and acyl chains, respectively. Both of these probes were very sensitive to CBD and 11-OH-Δ^9-THC (fig. 3) with significant increases in polarization occurring at 10^{-7} M concentrations. The probes do not, however, discriminate between CBD and 11-OH-Δ^9-THC and the effect of both drugs was similar at both depths monitored by these probes.

To describe further the interactions of the cannabinoids with the lipid component of cell membranes, phospholipid vesicles were used as simple models of cell membranes. The importance of bilayer composition and initial bilayer order in the effects of the cannabinoids were determined using vesicles composed of DPOPC (16:1), DMPC (14:0) or DPPC (16:0) (fig. 4). Both 11-OH-Δ^9-THC and CBD increased DPH fluorescence polarization in the unsaturated and relatively fluid DPOPC vesicles (fig. 4A). In contrast, both cannabinoids decreased fluorescence polarization of DPH in the more rigid, saturated DMPC (fig. 4B) and DPPC (fig. 4C) vesicles. In fact, both 11-OH-Δ^9-THC and CBD dramatically increased DPH mobility in the very rigid DPPC vesicles. From these studies, it appears that the

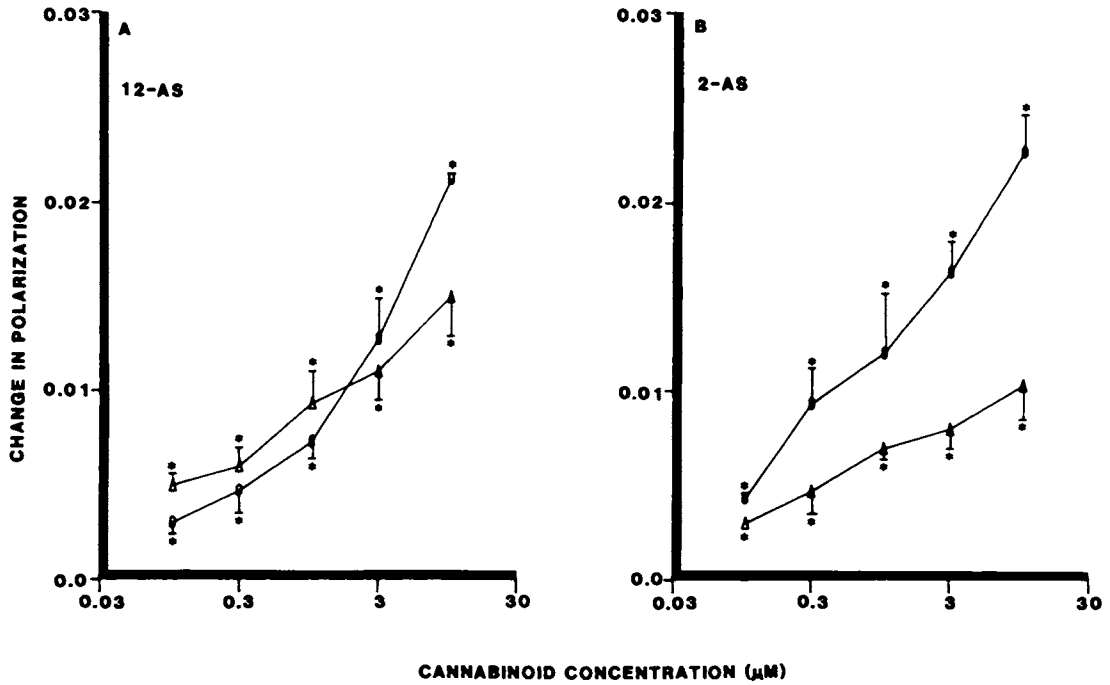

Fig. 3. Effects of cannabinoids on fluorescence polarization of 2-AS and 12-AS in total lipid extract of mouse brain SPM. Cannabinoids were dissolved in DMSO and added to vesicles prepared from a total lipid extract of mouse brain SPM-2. Fluorescence polarization of 12-AS (A) and 2-AS (B) were determined at 35°C. Each point is the mean of three determinations, vertical lines represent S.E.M. 11-OH-Δ^9-THC is represented by Δ, CBD is represented by O. Mean predrug polarization of 12-AS was 0.107 ± 0.02 and mean predrug polarization of 2-AS was 0.164 ± 0.003. * $P < .05$ compared to pretreatment polarization.

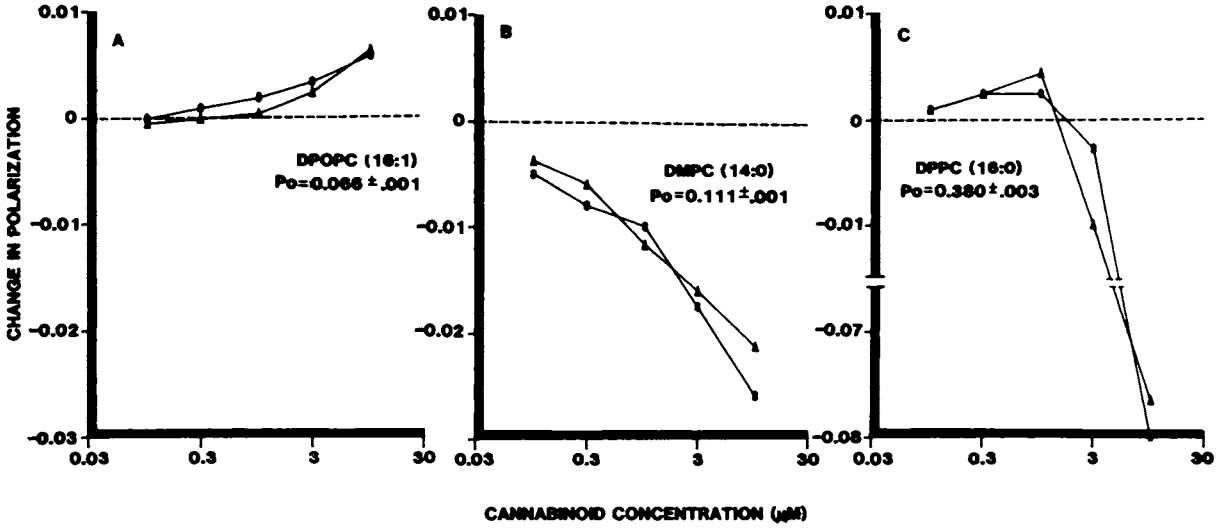

Fig. 4. Effects of cannabinoids on fluorescence polarization of DPH in PC vesicles. Vesicles were prepared using lipid indicated. Cannabinoids were dissolved in DMSO. Fluorescence polarization of DPH was determined at 35°C. Δ, 11-OH-Δ^9-THC; O, CBD. Po is the mean predrug polarization of DPH; shown ± S.E.M. A, DPOPC vesicles: $n = 3$ for both cannabinoids. B, DMPC vesicles: $n = 2$ for CBD and $n = 3$ for 11-OH-Δ^9-THC. C, DPPC vesicles: $n = 4$ for both cannabinoids.

direction and degree of change in DPH mobility produced by these drugs is dependent upon the degree of initial bilayer order. The cannabinoids increase DPH mobility in rigid bilayers and decrease DPH mobility in fluid bilayers.

The effects of the cannabinoids on the liquid-crystalline and solid phases of the same lipid were measured using temperature scans of DPPC vesicles (fig. 5). The polarization of DPH in DPPC vesicles was determined from 52 to 29°C. In the presence of DMSO alone, the phase transition is very sharp which is characteristic of a homogeneous bilayer. Both 11-OH-Δ^9-THC and CBD decreased the phase T_m and broadened the phase transition. These results indicate that the drugs disorder bilayer structure and decrease the cooperativity of the lipids. In addition, both drugs decreased DPH fluorescence polarization below T_m and increased it above T_m, which is consistent with the finding that the cannabinoids increase probe mobility in the solid phase and decrease probe mobility in the liquid-crystalline phase.

The influence of CHOL on the effects of the cannabinoids was determined in egg yolk PC and DMPC vesicles (figs. 6 and 7). In vesicles of egg yolk PC alone, all drugs increased polarization of DPH (fig. 6A). Egg yolk PC has a large proportion of

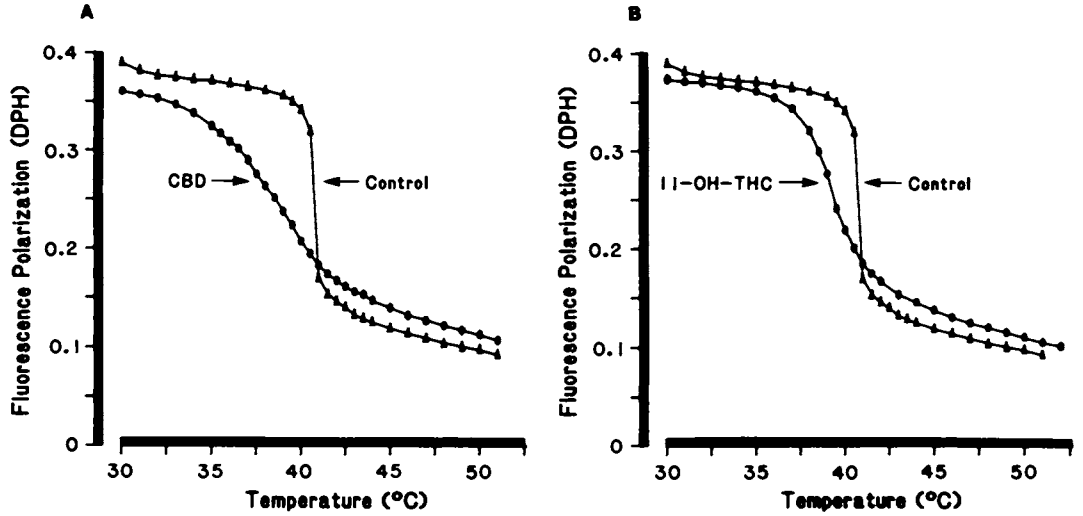

Fig. 5. Effects of cannabinoids on temperature scans of fluorescence polarization of DPH in DPPC. Vesicles were prepared from DPPC. Fluorescence polarization of DPH was determined from 52 to 29°C (cooling scans were used). △, scan in the presence of DMSO alone; ○, scan in the presence of 5 μM CBD (A) or 5 μM 11-OH-Δ^9-THC (B). Each point is the mean of two determinations.

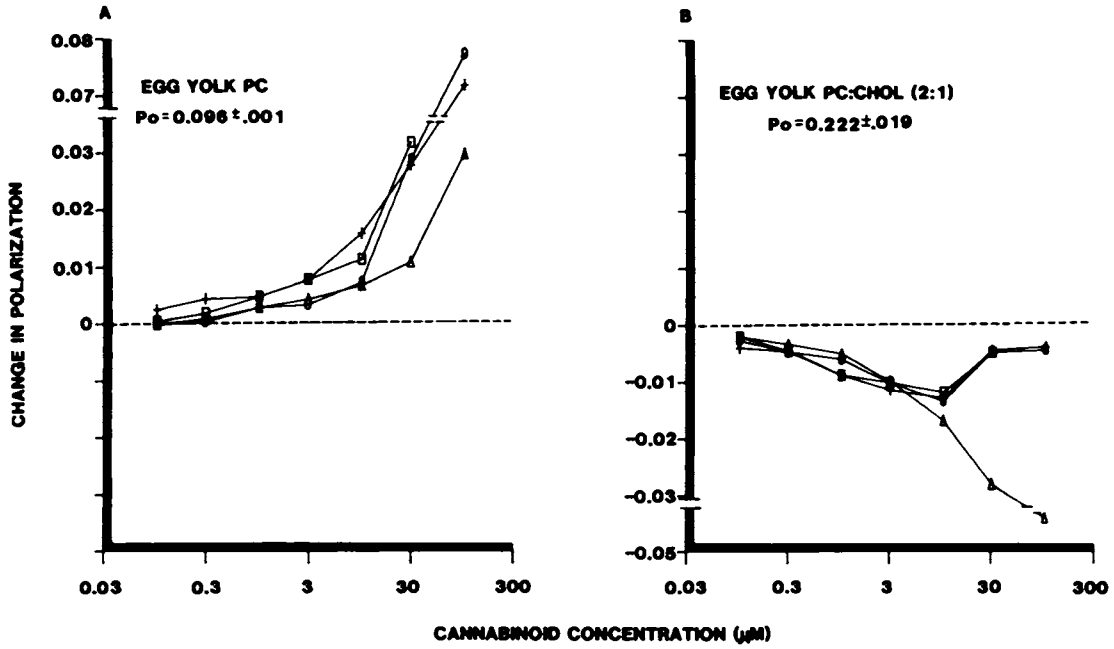

Fig. 6. Effects of the cannabinoids on fluorescence polarization of DPH in egg yolk PC vesicles in the absence or presence of cholesterol. Vesicles were prepared using (A) egg yolk PC alone or (B) with cholesterol (egg yolk PC-CHOL, 2:1). The cannabinoids were dissolved in DMSO. Fluorescence polarization of DPH was determined at 35°C. Po is the mean predrug polarization, shown ± S.E.M. Each point is the mean of two determinations. △, 11-OH-Δ^9-THC; ○, CBD; +, CBN; □, Δ^9-THC.

unsaturated fatty acyl chains and forms relatively fluid bilayers. None of the cannabinoids had effects on this bilayer at low concentrations but CBD and CBN produced large effects at 100 μM concentrations. In contrast, all of the drugs decreased polarization in the more rigid, CHOL-containing, egg yolk PC vesicles (fig. 6B). The cannabinoids were quite potent with appreciable decreases in polarization occurring at 0.3 to 1.0 μM. Interestingly, CBD, CBN and Δ^9-THC produced maximal decreases in DPH polarization at 10 μM concentrations and returned toward base line at higher concentrations. However, 11-OH-Δ^9-THC decreased polarization in a concentration-related, monophasic manner.

Although DMPC vesicles are only slightly more rigid than egg yolk PC vesicles, the cannabinoids decreased DPH polarization in DMPC vesicles (fig. 7A). This is the opposite of their effects on egg yolk PC vesicles and demonstrates that lipid unsaturation may be an important determinant of cannabinoid action. Addition of CHOL to the DMPC vesicles did not greatly alter the effects of the drugs (fig. 7B).

The results of the studies presented are summarized in table 1. Also reported in table 1 are the correlation coefficients that result when base-line polarization (*i.e.*, initial membrane or vesicle rigidity) is compared to the change in polarization produced by 10 μM concentrations of CBD or 11-OH-Δ^9-THC. Correlation coefficients (r^2) of 0.33 and 0.41 for 11-OH-Δ^9-THC and CBD, respectively, indicate that initial membrane or vesicle order can account for only 30 to 40% of the variance in drug action among the preparations.

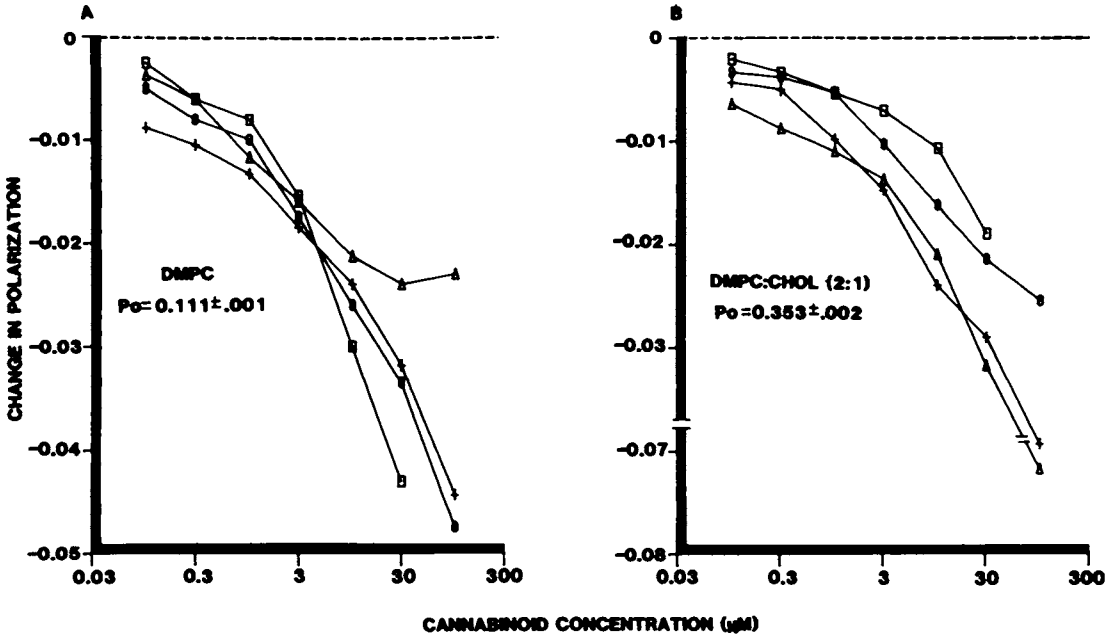

Fig. 7. Effects of the cannabinoids of fluorescence polarization of DPH in DMPC vesicles in the absence or presence of cholesterol. Vesicles were prepared using (A) DMPC alone or (B) with cholesterol (DMPC-CHOL, 2:1). The cannabinoids were dissolved in DMSO. Fluorescence polarization of DPH was determined at 35°C. Po is the mean predrug polarization, shown ± S.E.M. Δ, 11-OH-Δ^9-THC; O, CBD; +, CBN; □, Δ^9-THC. A, $n = 4$ for CBN, $n = 2$ for CBD and Δ^9-THC and $n = 3$ for 11-OH-Δ^9-THC; B, $n = 3$ for CBD, CBN and 11-OH-Δ^9-THC and $n = 2$ for Δ^9-THC.

TABLE 1

Summary of effects of cannabinoids on fluorescence polarization (P) of probe molecules in membrane and lipid vesicles

Probe and Vesicle Type	Base-line Polarization[c]	11-OH-Δ^9-THC		CBD	
		Effect on P	Conc. ΔP 0.01[a]	Effect on P	Conc. ΔP 0.01[a]
DPH probe					
SPM	0.260 ± 0.001	↓	4	↑	25
SPM lipid extract	0.252 ± 0.004	↓	10	↑	30
DMPC	0.111 ± 0.001	↓	0.8	↓	0.8
DMPC + CHOL (2:1)	0.313 ± 0.002	↓	0.6	↓	3
EPC[b]	0.096 ± 0.001	↑	30	↑	12
EPC + CHOL (2:1)	0.251 ± 0.003	↓	3	↓	0.8
DPOPC	0.066 ± 0.001	↑	>10	↑	>10
DPPC	0.380 ± 0.003	↓	3	↓	1.5
EPE[b] + PS (2:1)	0.106 ± 0.002	0		↑	>30
DPPE + PS (2:1)	0.317 ± 0.001	↓	>30	↓	8
TMA-DPH probe					
DMPC + CHOL (2:1)	0.326 ± 0.001	↓	11	↓	2
2-AS probe					
SPM lipid extract	0.164 ± 0.003	↑	10	↑	0.4
12-AS probe					
SPM lipid extract	0.107 ± 0.020	↑	2	↑	2

[a] Concentration (micromolar) required to reduce polarization by 0.01.
[b] EPC, egg yolk PC; EPE, egg yolk PE.
[c] The r^2 for base-line polarization *vs.* the change in polarization produced by 10 μM concentrations of 11-OH-Δ^9-THC was 0.328 and of CBD was 0.405.

The outer leaflet of the SPM bilayer is largely made up of PC-containing phospholipids whereas the inner (cytoplasmic) leaflet is enriched in PE and PS phospholipids (Fontaine *et al.*, 1980). As a model of the inner leaflet of the cell membrane, vesicles were made from a mixture of the egg yolk PE and brain PS. Both lipids are highly unsaturated and the vesicles formed were relatively fluid (fig. 8A). Neither CBD nor 11-OH-Δ^9-THC had any significant effect on these vesicles except that CBD increased DPH polarization slightly at a concentration of 30 μM. To determine whether the drugs would decrease order in a rigid PE-PS vesicle, DPPE and brain PS vesicles were examined (fig. 8B). In these vesicles, CBD decreased DPH polarization but 11-OH-Δ^9-THC had no effect. These data suggest that the inner leaflet of membrane bilayers would be quite insensitive to the cannabinoids, especially 11-OH-Δ^9-THC.

TMA-DPH is a DPH derivative that, due to its quarternary nitrogen, is anchored at the membrane surface (Prendergast *et al.*, 1981). To compare the effects of the cannabinoids on membrane order at the surface and in the core, their effects on TMA-DPH and DPH polarization were measured in DMPC-CHOL vesicles (fig. 9). As was shown above, both 11-OH-Δ^9-THC and CBD decrease the fluorescence polarization of DPH, with 11-OH-Δ^9-THC being more potent (fig. 9A; table 1). Both cannabinoids also decreased polarization of TMA-DPH; however, CBD was more potent than 11-OH-Δ^9-THC (fig. 9B; table 1). CBD was about equipotent in its effects on DPH and TMA-DPH, indicating that it perturbs the membrane surface and

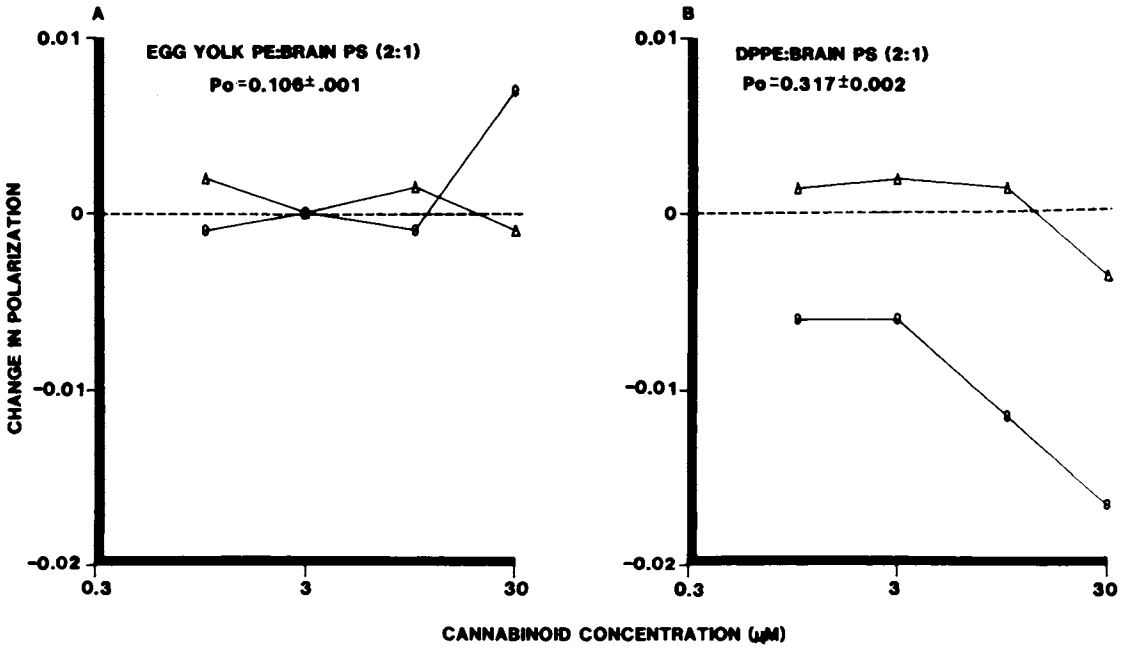

Fig. 8. Effects of the cannabinoids on fluorescence polarization of DPH in PE and PS vesicles. Cannabinoids were dissolved in DMSO and added to vesicles prepared from (A) brain PE-egg yolk PS (2:1) or from (B) DPPE-egg yolk PS (2:1). The mean predrug polarization (Po) is shown ± S.E.M. Each point is the mean of two determinations. △, 11-OH-Δ^9-THC; ○, CBD.

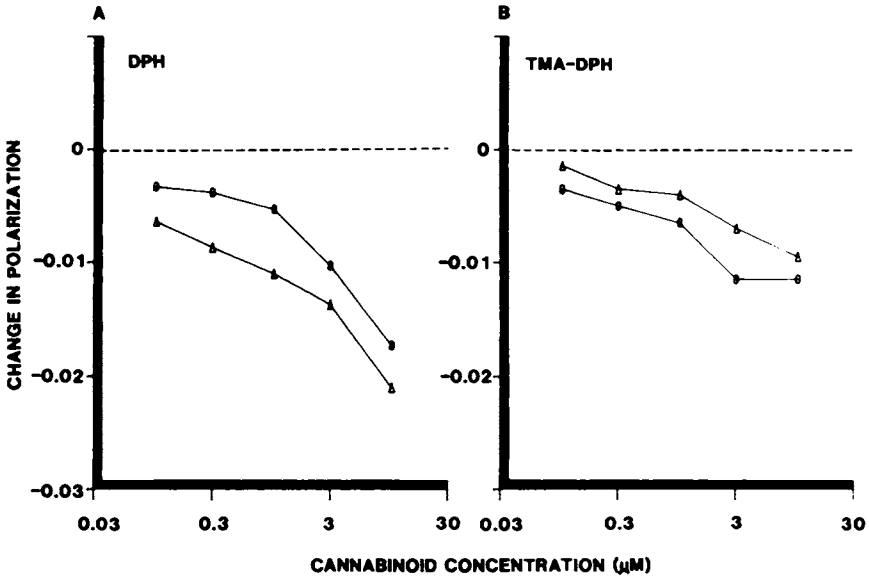

Fig. 9. Effects of the cannabinoids on fluorescence polarization of DPH or TMA-DPH in DMPC-CHOL vesicles. Vesicles were made from DMPC and cholesterol (2:1) and fluorescence polarization of DPH or TMA-DPH was determined at 35°C. The cannabinoids were added in DMSO. A, effects of cannabinoids on DPH polarization; mean predrug polarization was 0.313 ± 0.002. For 11-OH-Δ^9-THC (△), n = 3 and for CBD (○), n = 4. B, effects of the cannabinoids on TMA-DPH polarization; mean predrug polarization was 0.326 ± 0.001. For both 11-OH-Δ^9-THC (△) and CBD (○), n = 2.

core similarly. In contrast, 11-OH-Δ^9-THC produced less effect on TMA-DPH than on DPH, indicating less action on the membrane surface than on the membrane core.

According to the Perrin equation, a change in polarization of probe fluorescence can occur as a result of either a change in probe mobility or a change in the lifetime of the excited state of the probe (Shinitzky and Barenholz, 1978). The effects of the cannabinoids on fluorescence lifetime (τ) of DPH in SPM are shown in table 2. DMSO produced a small and statistically insignificant decrease in fluorescence lifetime. 11-OH-Δ^9-THC and CBD (at 10 μM concentrations) slightly decreased and increased the fluorescence lifetime of DPH, respectively. Neither change was significantly different from the small change produced by DMSO.

Discussion

The cannabinoids produced complex changes in the fluorescence polarization of DPH in brain synaptic plasma membranes. Both Δ^9-THC and 11-OH-Δ^9-THC decreased DPH fluorescence polarization at low concentrations (1–3 μM) whereas CBN produced a similar change at a 3- to 10-fold higher concentration. CBD did not decrease DPH fluorescence polarization at any concentration. The synaptic membrane disordering potency of these four cannabinoids (as measured by DPH mobility) correlates to some extent with their reported psychoactive potency in humans (Hollister 1974; Perez-Reyes *et al.*, 1973) and with the degree of their generalization to Δ^9-THC in drug discrimination paradigms (Jarbe *et al.*, 1977; Browne and Weissman, 1981). In particular, CBN has been

TABLE 2

Effects of 11-OH-Δ^9-THC and CBD on DPH fluorescence lifetime in rat brain SPM

Treatment[a]	τ		$\Delta\tau$
	Before treatment	After treatment	
	nsec		nsec
DMSO[b]	9.42 (0.42)[c]	9.29 (0.40)	−0.13 (0.053)
11-OH-Δ^9-THC (10 μM)	9.02 (0.43)	8.75 (0.42)	−0.27 (0.09)
CBD (10 μM)	8.94 (0.50)	9.00 (0.53)	+0.06 (0.03)

[a] DMSO or cannabinoids in DMSO were added to cuvettes after initial lifetime determination. For DMSO and CBD, $n = 3$ and for 11-OH-Δ^9-THC, $n = 4$.
[b] DMSO equivalent to that added with cannabinoids (1.5 μl).
[c] S.E.M.

shown to be approximately 10-fold less potent than Δ^9-THC in its ability to produce tachycardia and the feeling of "high" in humans (Perez-Reyes *et al.*, 1973) and its ability to mimic the discriminative stimuli produced by Δ^9-THC in rats (Browne and Weissman, 1981). The effects of Δ^9-THC and its metabolite 11-OH-Δ^9-THC diverge at suprapharmacological concentrations although these two cannabinoids are behaviorally similar. This may be due to differences in the physiochemical and pharmacokinetic properties of these compounds (Paton, 1975). Δ^9-THC is rapidly converted to the 11-OH metabolite in humans and animals and the onset of the high in humans after Δ^9-THC injection is more closely related to blood levels of 11-OH-Δ^9-THC than the parent compound (Harris *et al.*, 1977), which suggests that the effects of 11-OH-Δ^9-THC may be more relevant to the mechanism of psychoactivity of marijuana.

DPH is a nonpolar molecule which preferentially distributes through the center of the membrane bilayer (Lentz *et al.*, 1976). At a constant temperature, the fluorescence polarization of DPH (or of any probe molecule) is directly related to the mobility of the probe and inversely related to the excited state lifetime of the probe (Shinitzky and Barenholz, 1978). In our experiments, the cannabinoids had only slight effects on fluorescence lifetime and the effects were the opposite of what would be expected if the observed changes in polarization were due to changes in fluorescence lifetime. Therefore, the effects of the cannabinoids on fluorescence polarization reflect changes in the mobility of the probe. The motion of the DPH molecules is greatly influenced by the ordering of the phospholipid acyl chains and the depolarization of fluorescence emission appears to be due to DPH "wobbling" about axes that are perpendicular to the membrane surface (Kinosita *et al.*, 1977). The decrease in DPH fluorescence polarization produced by Δ^9-THC and 11-OH-Δ^9-THC in this study indicates that these cannabinoids disorder or fluidize the central region of SPM and vesicles formed from SPM lipids. Conversely, high concentrations of Δ^9-THC, CBD and CBN increase membrane acyl chain order.

It is difficult to correlate *in vitro* cannabinoid concentrations with *in vivo* doses. Our tracer studies suggest that low micromolar concentrations *in vitro* are relevant to effects produced by moderate (3–6 mg/kg) cannabinoid i.v. doses in rodents. Furthermore, many of the effects of Δ^9-THC on membrane-associated proteins occur at low micromolar concentrations (Hillard and Bloom, 1982; Lindamood and Colasanti, 1980; Bloom *et al.*, 1978; Hershkowitz *et al.*, 1977). Therefore, the psychoactive cannabinoids disorder synaptic membranes in a concentration range that coincides with their effects on the functioning of several membrane proteins and with estimates of rodent brain drug concentration during cannabinoid intoxication. The effects of 3 μM Δ^9-THC and 11-OH-Δ^9-THC on DPH mobility are small but similar to the magnitude of effects produced by 0.1 to 0.3 mM pentobarbital and 80 mM ethanol (Harris and Schroeder, 1982). Small changes in membrane order (as monitored by DPH polarization) can produce large changes in membrane function (Pang *et al.*, 1979). Thus, the effects of low concentrations of the psychoactive cannabinoids on SPM phospholipid ordering observed in the present study are consistent with the hypothesis that the cell membrane is a primary site of cannabinoid action.

The physiological relevance of the effects produced by high (30 μM) concentrations of the cannabinoids is questionable. Extrapolation from our tracer studies would suggest that cannabinoid doses required to give rodent brain concentrations of 30 μM would be toxic. Furthermore, Hershkowitz and coworkers (1977) found that 50 μM Δ^9-THC caused morphological damage in synaptosomal preparations. Despite their possible pharmacological irrelevance, the effects produced by high Δ^9-THC concentrations are interesting as biphasic concentration relationships have been reported for *in vitro* Δ^9-THC effects on several membrane-mediated processes, including ligand receptor binding (Hillard and Bloom, 1982) and neurotransmitter uptake systems (Hershkowitz *et al.*, 1977; Poddar and Dewey, 1980).

In contrast to its effects on polarization of DPH, 11-OH-Δ^9-THC increased fluorescence polarization of both 2-AS and 12-AS in extracted SPM lipids. CBD had similar effects. There are several possible explanations for the difference in 11-OH-Δ^9-THC effects on the stearic acid probes and DPH. First, the orientation of the emission dipoles of AS and DPH are different, so they detect different types of phospholipid motion (Thulborn, 1981). DPH is sensitive to wobbling motions and acyl chain packing whereas the AS probes primarily monitor rotational movements about axes normal to the plane of the bilayer. Our results suggest that 11-OH-Δ^9-THC decreases phospholipid rotation in the upper and middle portions of the bilayer but disorders the bilayer core, as DPH mobility is increased. In contrast, CBD both decreases phospholipid rotational movements and increases ordering in the bilayer core. A second and perhaps more likely explanation for the difference between 11-OH-Δ^9-THC effects on 12-AS and DPH polarization is that, although the emission dipoles are located at about the same membrane depth (Kutchai *et al.*, 1983), the two probes are not distributed in the same manner. In biological membranes, lipids are distributed asymmetrically and may segregate into rigid and fluid domains within each bilayer (Karnovsky *et al.*, 1982). It is likely that the cannabinoids have distinct effects on different membrane domains. DPH distributes fairly evenly among the domains of the membrane (Thulborn, 1981), so the overall membrane perturbant effects detected by DPH will represent an average of the distinct effects of the cannabinoids on individual membrane domains. One consequence of this is that cannabinoid effects on specific domains may be underestimated which could explain the small magnitude of the change in DPH polarization in SPM produced by Δ^9-THC. In contrast, 12-AS is localized preferentially in the most fluid domains of membranes (Thulborn, 1981). It is possible (and is corroborated by the vesicle studies discussed below) that the cannabinoids increase membrane order in fluid domains and that only this

perturbant action is detected by 12-AS. This reasoning might suggest that the effects of the cannabinoids in rigid domains are more important for psychoactivity than their effects in fluid domains. A third explanation for these results is that the probes themselves perturb lipid ordering and that the cannabinoids can counteract this perturbation. This is unlikely because the lipid phase transitions are not altered by the AS probes (Thulborn, 1981).

In the second set of experiments described in this report, we have used homogeneous phospholipid bilayers as models of synaptic plasma membrane domains to elucidate further the interactions of the cannabinoids with biological membranes. Overall, these studies allow several generalizations to be made. First, the effects of the cannabinoids on brain SPM and SPM lipids cannot be mimicked by any simple binary mixture of lipids. Where 11-OH-Δ^9-THC and CBD have very different effects on SPM and SPM extracted lipids, they have qualitatively similar effects in all of the bilayers that were examined. A second generalization that can be made is that the effects of the cannabinoids vary markedly with bilayer rigidity. The results of the studies of 11-OH-Δ^9-THC and CBD interactions with DPOPC, DMPC and DPPC vesicles, along with temperature scans of DPPC vesicles, suggest that both cannabinoids increase DPH mobility in rigid, gel phase lipids and decrease DPH mobility in fluid, liquid-crystalline phase lipids. Overall, the cannabinoids were more efficacious and more potent at decreasing bilayer order than at increasing bilayer order. However, the drugs only increased membrane order in bilayers of unsaturated phospholipids, suggesting that initial membrane rigidity is not the only important determinant of cannabinoid action. Thirdly, we found that the PE-PS rich regions of the membrane inner leaflet would be relatively insensitive to the cannabinoids, especially 11-OH-Δ^9-THC.

Although these results are the first demonstration of the interactions of the cannabinoids with biological membranes, other investigators have demonstrated cannabinoid effects in lipid bilayers using several techniques. Using ESR, Lawrence and Gill (1975) found that Δ^9-THC increased the mobility of a stearic acid ESR probe in egg yolk PC-CHOL (1.0:0.9) vesicles whereas CBD and CBN decreased probe mobility. Pang and Miller (1978) also reported that CBD decreased probe mobility in egg yolk PC-CHOL vesicles. In our studies, DPH mobility was increased by all the cannabinoids in PC-CHOL vesicles. Recent NMR studies of Δ^9-THC found a biphasic decrease in membrane order in egg yolk PC-CHOL (2:1) vesicles whereas CBD increased membrane order. Thus, ESR and NMR studies of artificial membranes are in good agreement with our fluorescence studies of synaptic membranes. For lipid vesicles, our results with Δ^9-THC agree with those from ESR and NMR, but our results with CBD are not consistent with ESR and NMR data.

In summary, these results indicate that the hydrophobic core of brain synaptic membranes is sensitive to the disordering effects of the psychoactive cannabinoids Δ^9-THC and 11-OH-Δ^9-THC and at higher concentrations, CBN. To our knowledge, this is the first demonstration of cannabinoid perturbation of biological membranes. Our studies in model lipid bilayers confirm the membrane perturbant activity of the cannabinoids but none of the model membranes that we used responded to the cannabinoids in the same manner as the synaptic membranes. The membrane perturbant activity of the cannabinoids occurs at low micromolar concentrations and could be responsible for the effects of the drugs on membrane associated proteins. However, the role of the membrane disordering effects of the cannabinoids in the marijuana high has yet to be determined. Overall, these studies support the hypothesis that the cell membrane phospholipids can act as a selective cannabinoid recognition site and that perturbation of membrane phospholipid ordering is one mechanism of cannabinoid action.

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