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COMMUNICATIONS

Correlation Between the Psychotropic Potency of Cannabinoids and Their Effect on the ^{1}H-NMR Spectra of Model Membranes

Keyphrases □ Membranes—model, correlation between psychotropic potency of cannabinoids and their effect on the ^{1}H-NMR spectra □ Cannabinoids—correlation between psychotropic potency and their effect on the ^{1}H-NMR spectra of model membranes □ Cholesterol—model membranes, correlation between the psychotropic potency of cannabinoids and their effect on the ^{1}H-NMR spectra □ Phosphatidylcholine—model membrane, correlation between the psychotropic potency of cannabinoids and their effect on the ^{1}H-NMR spectra

To the Editor:

Little is known of the mode of psychotropic action of Δ^1-tetrahydrocannabinol (I), the major active component of hashish (1). It has been suggested that this drug, as well as other psychotropic cannabinoids, exerts its psychotropic effect through a nonspecific interaction with lipid constituents of nerve cell membranes (2). To investigate such nonspecific interactions, phospholipid vesicles (liposomes) have been used as model membranes. These models represent an oversimplification of the complex biological membranes. However, the possibility of manipulating their composition and size permits deduction of the relative importance of the various structural and compositional factors of the membrane in determining the interactions of membranes with drugs. For the effect of a drug on the physical properties of a model membrane to be regarded as relevant to the mode of action of the drug, a correlation must be established between the effect of various derivatives of the drug and their potency on the membrane.

For psychotropically active cannabinoids, electron spin resonance measurements showed that these drugs reduce the order within the bilayer (3) when introduced into vesicles composed of phosphatidylcholine and cholesterol. The disordering effect of five different cannabinoids correlated qualitatively with their psychotropic potency. The psychotropically nonactive drug, cannabidiol (VI), increased the order parameter within the bilayer (3). Since electron spin resonance, as well as several other physicochemical techniques, involve the use of external probes, which might alter the bilayer properties, we have recently studied the effect of the cannabinoids, I and VI, on the ^{1}H-NMR spectra of lipid vesicles (4). The conclusions of this research were similar to those obtained from the electron spin resonance study: addition of small amounts of I (1 mole percent) to vesicles composed of egg phosphatidylcholine and cholesterol (2:1) caused narrowing of the apparent linewidth of the phospholipid methylene groups signal, whereas the chemically similar, nonactive compound, VI, had the opposite effect.

The purpose of the present work was twofold. First, we wanted to establish a correlation between the potency of various cannabinoids[1] and the effect of small quantities (1 mole percent) of these drugs on the apparent linewidth of the NMR resonance of the phospholipid hydrocarbon chain protons in model membranes composed of egg phosphatidylcholine and cholesterol. Second, in a previous work we showed that in the absence of cholesterol in the model membranes, small amounts of I do not produce any significant change in the linewidth of the ^{1}H-NMR resonances. Therefore, we found it of interest to study the role of cholesterol in the interactions of I with the model membranes. These interactions most likely are not due to the existence of specific interactions of I with cholesterol, since there is no evidence for such interactions in the ^{1}H-NMR spectrum of a mixture of these two components in chloroform (4). One alternative explanation was that the lack of a fluidizing effect in the absence of cholesterol is due to the disrupted packing in the highly curved small unilamellar vesicles (5–7). More specifically, vesicles formed by sonification of mixtures of phosphatidylcholine and cholesterol are larger and less curved than those made of the pure phospholipid by the same method. This may cause the difference between the effect of I on vesicles with and without cholesterol. To investigate this possibility, we studied the effect of cannabinoids on the larger vesicles (~500-Å diameter) made of pure phosphatidylcholine by the French press method (8). The results of this study, in conjunction with the dependence of the fluidizing effect of I on the cholesterol content in the vesicles, indicate that cholesterol is important in the interaction of this drug with model membranes.

The model membranes were prepared as follows: solutions of phosphatidylcholine[2] and cholesterol[3] in chloro-

[1] All cannabinoids used in this study (Table I) were a gift of Professor R. Mechoulam of the Natural Products Laboratory, Hebrew University.

[2] Egg yolk phosphatidylcholine (lecithin, grade 1) was purchased from Makor Chemicals (Jerusalem). It was at least 99% pure, giving one spot on a TLC plate at a 1-μmole loading. It was used without further purification.

[3] Cholesterol (Merck) was recrystalized from ethanol.

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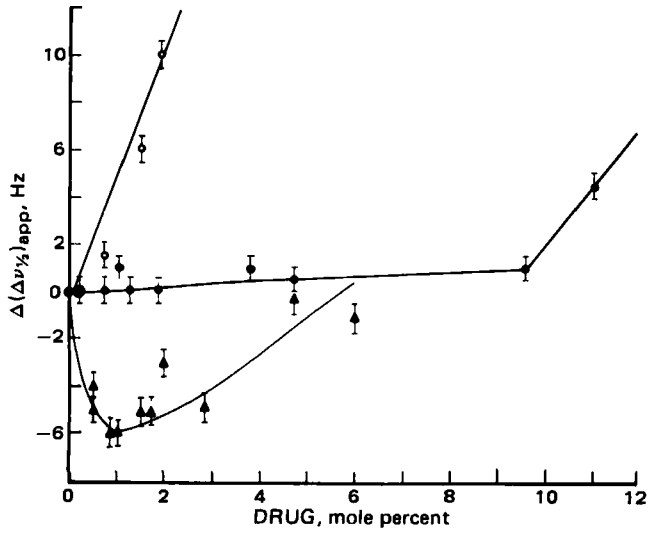


Figure 1—*The effect of I on the apparent linewidth of the phosphatidylcholine methylene signals $(\Delta\nu_{1/2})_{app}$ in vesicles made by the French press methoid (○) and by sonication with (▲) and without (●) cholesterol (33 mole percent). In the absence of cannabinoids, $(\Delta\nu_{1/2})_{app}$ was 22 Hz for the sonicated lecithin, 85 Hz for sonicated vesicles with cholesterol, and 35 Hz for the French press vesicles.*

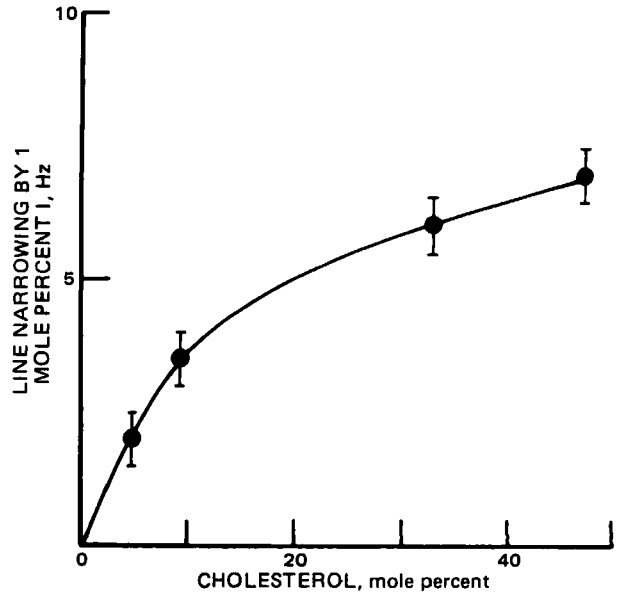


Figure 2—*The reduction in the apparent linewidth of the signal of phosphatidylcholine methylene protons introduced by 1 mole percent of I as a function of the cholesterol content of sonicated vesicles. Phosphatidylcholine concentration was 50 mg/ml.*

form were mixed at appropriate molar ratios, and the organic solutions were evaporated to dryness under nitrogen and then lyophilized. The dried mixtures were dispersed in deuterium oxide[4] to the proper concentration. Small unilamellar vesicles were prepared from these dispersions by ultrasonic irradiation[5] until clear dispersions were obtained. French press vesicles were prepared by a previous method (6)[6]. The final concentration of phosphatidylcholine for ^{1}H-NMR measurements was 50 mg/ml.

The drugs were dissolved in deuterated dimethyl sulfoxide[4], and their solutions were added to the prepared liposome dispersions to give the appropriate molar ratio of drug–phosphatidylcholine. ^{1}H-NMR spectra were recorded immediately after sonication[7]. All spectra were recorded without accumulation and under the same conditions of radio frequency (RF) and modulation. The ^{1}H linewidths ($\Delta\nu_{1/2}$) were measured using a spectral width of 10 Hz/cm and sweep rate of 1 Hz/sec and are expressed as the difference between their measured values and the width of the ^{1}H—O—^{2}H signal in the same spectrum. Each result is an average of at least four consecutive scannings under the same conditions of RF and modulation. The experimental errors, indicated in Figs. 1 and 2, represent the deviation of the results from their averages. Care was taken to ensure reproducibility in the base line determination. This was achieved by the use of the base line of the corresponding spectrum determined at a spectral width of 40 Hz/cm.

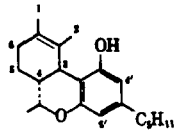
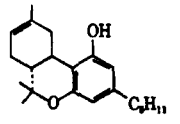
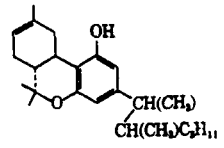
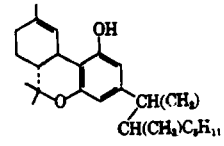
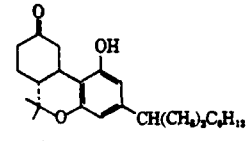
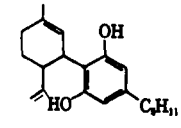
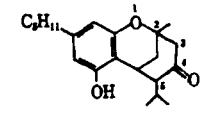
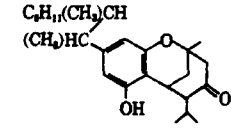
Figure 1 shows that addition of 1 mole percent of I to sonicated egg phosphatidylcholine vesicles does not change the apparent linewidth of the methylene group signal, whereas in cholesterol-containing vesicles, a small but reproducible narrowing is observed.

The effect of several other cannabinoids on the cholesterol-containing model membranes (Table I) shows a qualitative correlation between the apparent narrowing caused by the various cannabinoids and the psychotropic potency of these drugs. In spite of the difficulties in the interpretation of ^{1}H-NMR linewidths (5, 10, 11), we believe that this apparent narrowing is due to a drug-induced reduction of the restriction of the phospholipid molecular motions. However, other possibilities should also be considered. It may be thought that the signals of some protons are actually broadened by I beyond detection, which would result in an apparent narrowing of the bulk super Lorentzion methylene signal (12). This possibility can be ruled out, since the methylene signals studied are fully detected in all the spectra, as is evident from the constant ratio of intensities of the methylene–choline signals. It is also highly improbable that vesicle–vesicle interactions cause this line narrowing, as the opposite effect is expected from such interactions (5, 13). Thus, it may be concluded that in cholesterol-containing vesicles, I reduces the restriction of molecular motions of the phospholipid hydrocarbon chains. The correlation between the narrowing produced by various cannabinoids in lecithin cholesterol model membranes and the psychotropic potency of these drugs (Table I) supports the hypothesis that the reduction of molecular motion may be relevant to the psychotropic action of the cannabinoids. This is in agreement with an earlier conclusion (3), based on studies of electron spin resonance order parameters.

In an attempt to elucidate the role of cholesterol in this phenomenon, we carried out two additional experiments. First, we measured the effect of I on the apparent linewidth of pure phosphatidylcholine vesicles made by extrusion of the phospholipid through a French pressure cell (8). The latter vesicles are significantly larger than the extremely curved vesicles made by ultrasonic irradiation. Nonetheless, addition of I to the French press vesicles did not cause any narrowing of the proton signals (Fig. 1). This result is

[4] Deuterated solvents were purchased from Merck; deuterium oxide was 99.7% pure, $^2H_6$-dimethyl sulfoxide was 99% pure.
[5] Heat Systems 350W sonicator.
[6] Aminco French pressure cell, American Instrument Co.
[7] NMR measurements were done either on a Jeol JMN-100 spectrometer, operating at 100 MHz or on a Bruker WH-300 at 300 MHz.

Table I—Effects of Cannabinoid on the Apparent Linewidth of the Phosphatidylcholine Methylene Signal in Phosphatidylcholine–Cholesterol Vesicles [a] and their Psychotropic Activities [b]

		Dose, mg/kg	Activity	$\Delta\nu_{1/2}$, Hz
I (−)Δ^1-Tetrahydrocannabinol		0.05 0.1 0.5	+ ++ +++	−4.8
II (−)Δ^6-Tetrahydrocannabinol		0.1 0.5–0.3 1.0–2.0	● ++ +++	−1.5
III (−)Δ^6-Tetrahydrocannabinol dimethyl heptyl		0.05 0.1 1.0	++ +++ +++	−5.5
IV (+)Δ^1-Tetrahydrocannabinol dimethyl heptyl		—	—	+2.0
V Nabilone				+2.1
VI Cannabidiol		—	—	+2.3
VII Bezoxocin-4-one (2A)		5 10	—	+4.5
VIII Bezoxocin-4-one-dimethyl heptyl(2B)		0.25 0.5 1.0	— + +	−1.5

[a] ($\Delta\nu_{1/2}$) had the value of 85 ± 1.5 Hz for phosphatidylcholine–cholesterol (molar ratio 1:2) sonicated vesicles in the absence of cannabinoids but with 0.4% (v/v) dimethyl sulfoxide. $(\Delta\nu_{1/2})_{app}$ is the change in this parameter induced by various cannabinoids. [b] The psychotropic activity of the various compounds for rhesus monkeys as defined previously (9).

similar to that observed for the small vesicles of pure phosphatidylcholine and differs significantly from the result obtained upon addition of the drug to cholesterol-containing small vesicles. In another series of experiments, we studied the dependence of the line narrowing produced by 1 mole percent of I on the cholesterol content of small (sonicated) vesicles. As seen in Fig. 2, introduction of I results in a significant narrowing of the ^{1}H-NMR resonances, provided that the model membrane contains cholesterol.

The dependence of the line narrowing, produced by 1 mole percent of I on the cholesterol content of the membranes (Fig. 2), indicates that for cholesterol contents of ≥40 mole percent, the narrowing is maximal, and the drug reduces the motional restriction of the phospholipid paraffinic chains to the extent obtained at much lower cholesterol concentrations. This is in accord with the idea that the narrowing is not caused by a strong specific interaction with a well-defined stoichiometry between I and cholesterol. It is also consistent with the high-resolution NMR studies of mixed solutions of these components in chloroform (4). Nonetheless, the lack of line narrowing, in the absence of cholesterol in vesicles of larger sizes, suggests that the narrowing in cholesterol-containing vesicles (Fig. 1) is not due only to the tighter basal packing in these vesicles (6).

The role of cholesterol in the interaction of cannabinoid with the model membranes is not clear and will have to be investigated further. Nevertheless, the present results suggest that cannabinoids probably exert their psychotropic effect by altering the physical properties of the lipid matrix of membranes through a mechanism in which cholesterol plays an important role.

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Experimental Evidence for Concentration-Dependent Plasma Protein Binding Effects on the Apparent Half-Lives of Restrictively Cleared Drugs

Keyphrases □ Plasma protein binding—concentration dependent, effects on the apparent half-lives of restrictively cleared drugs □ Pharmacokinetics—experimental evidence for concentration-dependent plasma protein binding effects on the apparent half-lives of restrictively cleared drugs □ Disopyramide—experimental evidence for concentration-dependent plasma protein binding effects on the apparent half-lives of restrictively cleared drugs

To the Editor:

Several early publications have addressed the influence of concentration-dependent (nonlinear) plasma protein binding on the pharmacokinetics of drugs which undergo restrictive clearance (elimination is proportional to free, unbound drug in serum or plasma) (1–3). Relatively simple models which excluded the influence of tissue binding were used to simulate plasma concentrations of free drug (C_f) and of total (free plus protein bound) drug (C_t). More recently, these earlier studies were extended (4) to include models which consider tissue binding and drugs which undergo nonrestrictive clearance (elimination is proportional to total drug in plasma). It was reported that log concentration–time plots of the elimination phase of C_f and C_t for drugs which undergo restrictive clearance may be linear, concave, or convex, depending on the extent to which drugs are bound to plasma and tissue protein (4).

Experimental data verifying the results of these simulation studies in humans are difficult to obtain because of the absence of a model drug which demonstrates concentration-dependent plasma protein binding at plasma concentrations achieved following the administration of safe, therapeutic doses and the absence of an analytical method sensitive enough to accurately measure plasma concentrations for extended time periods following the administration of safe doses of the drug. This is because the behavior of the decay of plasma concentration–time plots may require a wide concentration range to be expressed fully.

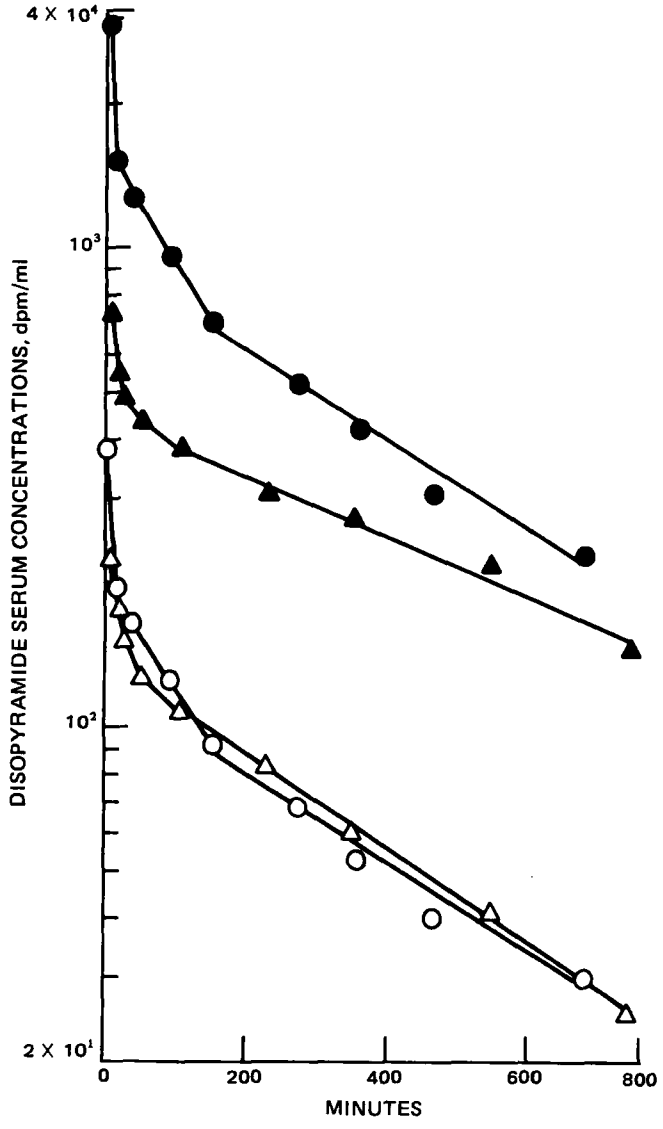

Figure 1—*Free (○,△) and total (●,▲) serum concentrations of [¹⁴C]disopyramide at various times following the administration of carbon 14 alone (○,●) and the simultaneous administration of [¹⁴C]disopyramide and the oral dose (△,▲). In each case a linear regression line was drawn through the last 5 serum concentration–time points.*

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