Biochemical Pharmacology. Vol. 25. pp. 1241–1244. Pergamon Press, 1976. Printed in Great Britain.

THE INTERACTION OF HASHISH COMPOUNDS WITH PLANAR LIPID BILAYER MEMBRANES (BLM)

DIANA BACH, AVRAHAM RAZ and RACHEL GOLDMAN
Laboratory of Membranes and Bioregulation, The Weizmann Institute of Science, Rehovot Israel

(*Received* 5 *September* 1975; *accepted* 8 *November* 1975)

Abstract—The interaction of hashish compounds with phosphatidyl choline or phosphatidyl serine black lipid membranes are investigated. Asymmetric distribution of cannabidiol across lecithin membranes generates a membrane potential and a decrease in electrical resistance of a transient nature. Symmetric distribution of the compounds results in the generation of membranes of low resistance in which no membrane potential or transient behaviour of resistance can be observed.

It is generally accepted that Δ^1-tetrahydrocannabinol (THC) is the major psychoactive component of hashish (cannabis) [1]. Relatively little is known about the other constituents of hashish, in particular of cannabidiol (CBD) which may constitute up to 40 per cent of cannabis extracts [2]. Exposure to THC and CBD leads to different physiological responses *in vivo* [3]. Both compounds do however affect similarly various membrane systems when isolated cells or organelles are exposed to the compounds under *in vitro* conditions. THC and CBD have been shown to lead to membrane stabilization against osmotic lysis [4–6], to inhibition of glucose efflux from preloaded erythrocytes [7], inhibition of cation uptake and ATPase activity in rat and human erythrocytes [8], induction of K^+ leakage from bull sperm [9] and leakage of enzymes from mitochondria and lysosomes [6, 10, 11]. Cannabinoids are lipid soluble neutral compounds with a very high membrane–aqueous solution partition coefficient [12]. All the above-mentioned studies point to an effect of hashish compounds on the structural and functional integrity of membranes. It was deemed of interest to study the interaction of CBD and THC with artificial phospholipid membranes since the phospholipid domain of biological membranes seems to be a logical candidate for an interaction with lipid-soluble compounds.

In the present study we have looked at the interaction of hashish compounds with black lipid membranes generated from either lecithin or phosphatidyl serine.

MATERIALS AND METHODS

Cannabinoids. Cannabidiol (white crystalline powder) and Δ^1-tetrahydrocannabinol, a brownish oil, were obtained from Makor Chemicals, Jerusalem, Israel. Both compounds were dissolved in freshly distilled ethanol at a concentration of about 0.1 M and kept at $-18°$. The THC solution only adsorbed light at wavelengths of 320–265 nm, with absorption peak at 280 nm, characteristic of the pure compound, indicating that the concentration of the brownish oxidation products was very low.

Phospholipids. Egg lecithin, chromatographically pure, was obtained from Applied Science Laboratories, State College, Pa., U.S.A. Dioleyl lecithin was a gift from Dr. R. Pagano, Carnegie Institution of Washington, Baltimore, Md. U.S.A., egg lecithin Grade I and phosphatidyl serine monosodium salt, Grade I were obtained from Lipid Products, Nutfield, England.

Experimental set-up. Black lipid membranes (BLM) were formed from either 1% lecithin or 0.5% phosphatidyl serine in *n*-decane, at a temperature of 25° for the lecithin membranes and 37° for the phosphatidyl serine membranes. The technique of BLM formation and the electrical set-up for measuring both resistance and membrane potential were as described previously [13]. The aqueous phase in the outer (27.5 ml) and inner (5 ml) compartments was 0.15 M NaCl or KCl (unbuffered). $CaCl_2$ at a concentration of 10^{-3} M was added in some of the experiments with phosphatidyl serine. The salts, all of analytical grade, were dissolved in water double-distilled over permanganate. Cannabinoids (from 5 to 25 μl) were added either to the outer grounded compartment after the membrane turned black (asymmetric membranes) or were added to both compartments before the generation of the membrane (symmetric membranes). Surface tension of aqueous solutions of cannabinoids was measured with a torsion balance—Federal Pacific (Newark, N.J., U.S.A.) equipped with a Wilhelmy plate. Concentrated solutions of cannabinoids (in ethanol) were injected into stirred 0.15 M NaCl or KCl solutions. The measurements were performed at 22°.

RESULTS

Both THC and CBD are poorly soluble in water. To get an indication as to the saturation concentration of cannabinoids in 0.15 M NaCl or KCl the effect of cannabinoid concentration on the surface tension of the salt solutions was studied. At a concentration of 4×10^{-6} M both CBD and THC reduce the surface tension of the solutions from 73 ± 1 to 64 ± 1 dyne/cm. The surface tension is not reduced any further even by a 3-fold increase in concentration of the cannabinoids. At a concentration of about 8×10^{-6} M white crystals appear in solution of both of the compounds. A THC solution with a concentration of 3×10^{-5} M has high turbidity, while at

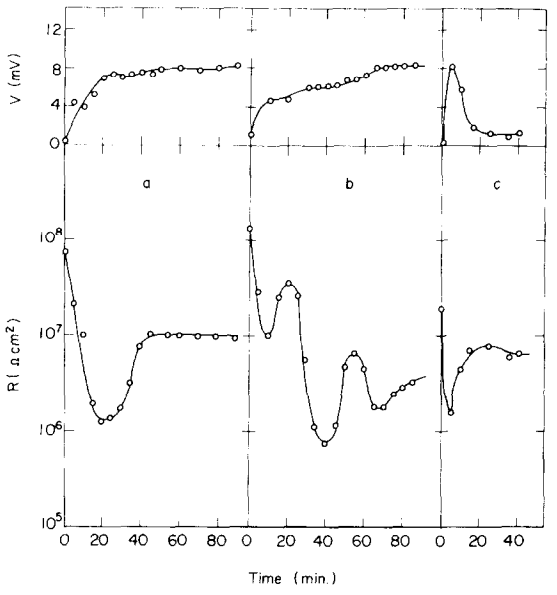

Fig. 1. The effect of cannabidiol on the d.c. resistance (R) and potential (V) of egg lecithin bilayer membranes. Cannabidiol was added at zero time (indicated in the figures) to one chamber only—(a) 0.15 M NaCl in both chambers; 2.2×10^{-5} M cannabidiol (b) 0.15 M NaCl in both chambers; 4.5×10^{-5} M cannabidiol (c) 0.15 M KCl in both chambers; 4.5×10^{-5} M cannabidiol. Each time course is representative of the behaviour of one membrane. Four different experiments have been carried out for each condition, giving essentially the same pattern.

the same concentration CBD has a crystalline appearance, and the crystals accumulate on the surface.

Exposure of black lecithin membranes to cannabidiol in the outer compartment, salt concentration in both compartments being equal, results in the appearance of an anionic potential (negative in the inner compartment) and a decrease in the electrical resistance. The peak value of the asymmetric potential is between -5 and -15 mV, the decrease in resistance is up to 100-fold and is transient. In the absence of cannabinoids the change of resistance with time was small (the lowest value obtained was $\sim 3.10^7 \Omega\,cm^2$) and the potential was less than -2 mV.

Figure 1 represents the time dependence of resistance and membrane potential of BLM exposed to cannabidiol. The time required to attain minimum membrane resistance depends on cannabidiol concentration and on the cation used. It is of interest that at lower cannabidiol concentrations (2.2×10^{-5} M, Fig. 1a) minimum resistance is reached at a time interval shorter than needed at higher concentrations (4.5×10^{-5} M, Fig. 1b and 9×10^{-5} M not shown), and the resistance reverts back to higher values. Membrane potential increases with time and reaches almost a stable peak value that is independent of cannabidiol concentration (Fig. 1a, b). Using KCl as the salt in the aqueous solution leads to similar transient resistance changes, but in contrast to the behaviour in NaCl solutions, membrane potential is also of a transient nature, its peak coinciding with the time at which resistance is at its minimum (Fig. 1c). Prior to the decrease in membrane resistance, the surround of the membrane becomes turbid, masking the lipid films. The turbidity decreases with time showing a

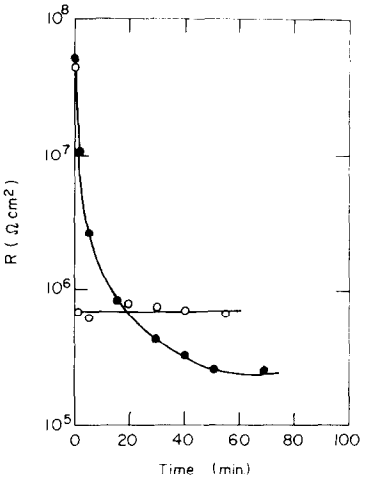

Fig. 2. Time dependence of resistance (R) of egg lecithin bilayers formed in 0.15 M NaCl containing cannabinoids on both sides (symmetric films). (○——○) 2×10^{-5} M cannabidiol; (●——●) 2.7×10^{-5} M Δ^1-THC. Each time course is a representative of the behaviour of one membrane. Four different experiments have been carried out for each condition, giving essentially the same pattern.

certain degree of correlation with the regaining of higher membrane resistance.

BLM generated in solutions containing either CBD or THC (symmetric membranes) have a very low resistance that remains constant at a low value for extended periods. There is no detectable membrane potential (Fig. 2).

THC, the psychoactive compound is a very potent membrane labilizer, BLM exposed to the compound are broken within minutes. At low concentrations $1\text{–}2 \times 10^{-5}$ M, membranes of longer life spans could be generated and decrease in resistance and generation of membrane potential were detected but the results were erratic. Symmetric membranes generated in the presence of THC were similar in their behaviour to those generated in the presence of cannabidiol (Fig. 2).

Negatively charged phospholipids are common constituents of biological membranes. The effect of cannabinoids on negatively charged BLM derived

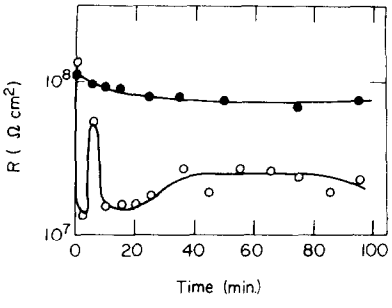

Fig. 3. Effect of cannabidiol 4.5×10^{-5} M on d.c. resistance (R) of phosphatidyl serine bilayers. Aqueous phase 0.15 M NaCl. (●——●) without Ca^{2+}; (○— —○) in the presence of 10^{-3} M Ca^{2+}. Cannabidiol was added at zero time to one chamber only. Each time course is a representative of the behaviour of one membrane. Four different experiments have been carried out for each condition, giving essentially the same pattern.

from phosphatidyl serine is given in Fig. 3. Cannabidiol does not significantly affect the electrical resistance of phosphatidyl serine membranes. In the presence of 10^{-3} M Ca^{2+}, cannabidiol gives up to a 10-fold decrease in membrane resistance. THC was very potent also in labilizing phosphatidyl serine membranes—the bilayers were stable for no more than 15 min during which period the effect of the compound was similar to that of CBD.

DISCUSSION

Membrane stabilization against osmotic lysis is commonly observed when membranes are exposed to low concentration of various lipid-soluble compounds. Upon exposing the membranes to higher concentrations of the lipid-soluble compounds a rapid labilization and lysis ensue. The stabilizing phase in the 'biphasic effect' exhibited by many drugs is usually indicative of an interaction between the drug and the membrane that confers on the membrane a higher expansion capacity under hypotonic conditions. Membrane labilization under high concentrations of the drug is in line with an extensive interference with phospholipid organization within the membrane [14].

Measurements of electrical resistance of phospholipid membranes are directed at a different membrane parameter, namely permeability to ions. In the present study we have shown that cannabidiol and possibly also THC interact with lecithin membranes in a way leading to a decrease in the electrical resistance. The generation of an anionic membrane potential in the absence of salt gradient suggests that an anionic species traverse the membrane creating an asymmetry. The decrease in resistance is transient to a certain degree when CBD is applied on one side of a BLM implying that the drug is able to diffuse across the membrane tending to equilibrate its concentration on both sides. Another possibility is reorganization within the membrane of cannabinoid aggregates that interact with it. The primary aggregates may affect membrane permeability to a higher extent than the redistributed cannabinoid. In fact the cloudy appearance of the BLM at the time of minimum resistance, and the clearing of the membrane at the phase of reversion in resistance to a higher value lends some support to such a mechanism. The transient nature of the effect of the cannabinoids may in addition stem from a possible equilibration of the compound with the torus of the membrane, with phospholipid suspended in solution (remains of the lipid applied to the support) and possibly also from a rate-determining step involving the transport of the compound across the unstirred layers at the membrane–solution interface.

A similar transient decrease in resistance was found by Van Zutphen *et al.* [15], and Seufert [16, 17] in response to addition of various detergents on one side of the bilayer membranes. Seufert [16, 17] has also observed the generation of an anionic potential upon interaction of anionic and nonionic detergents in the absence of a salt gradient.

In negatively charged bilayers CBD has no effect on membrane resistance suggesting that it is a negative entity that permeates the modified membrane and creates the potential. Addition of Ca^{2+} ions leads to a certain masking of the negative charges on the surface and probably enables negative entities, be it free chloride ions or any interacting species of chloride ions with CBD to reach the surface and penetrate the membrane. McLaughlin *et al.* [18] found simular effects of surface charge and its reduction by Ca^{2+}, on the transport of negatively charged iodide across phosphatidyl serine or phosphatidyl glycerol bilayers.

The observation that the time needed for the membrane to reach its minimal resistance increases with increasing CBD concentration in solution (Fig. 1) could evolve from the tendency of CBD to form crystals at concentrations above 8×10^{-6} M. Under the experimental conditions used, higher concentrations could mean bigger aggregates or crystals and thus a longer time needed for interaction with the membranes.

The transient nature of the decrease in membrane resistance is relevant to hashish interaction with biological systems. It was found that its interaction with erythrocytes is reversible [5] namely the compound can leave the membrane without causing a permanent change in structure. The fact that CBD or THC can readily leave the membrane results in a rapid redistribution of these compounds. In living cells, such redistribution means that the compound would get access to intracellular membranes and other structures. The effect of either THC or CBD on intracellular organelles is not necessarily reversible [6], the extent of damage depending on the concentration of the compounds at the intracellular site. Irreversible damage leading to extensive cell vacuolation, autophagocytosis and eventually to cell death was indeed observed when mouse peritoneal macrophages were exposed to either THC or CBD [19] and in alveolar macrophages derived from hashish smokers [20].

Acknowledgement—We should like to thank Professor I. R. Miller for his instructive interest in this work.

REFERENCES

1. R. Mechoulam, *Science, N.Y.* **168,** 1159 (1970).
2. L. Grlić, *Bull. Narcotics* **14,** 37 (1962).
3. M. Ten Ham and Y. De Jong, *Psychopharmacologia* **41,** 169 (1975).
4. E. Alhanaty and A. Livne, *Biochim. biophys. Acta* **339,** 146 (1974).
5. A. Raz, A. Schurr and A. Livne, *Biochim. biophys. Acta* **274,** 269 (1972).
6. A. Raz, A. Schurr, A. Livne and R. Goldman, *Biochem. Pharmac.* **22,** 3129 (1973).
7. A. Schurr, N. Sheffer, Y. Graziani and A. Livne, *Biochem. Pharmac.* **23,** 2005 (1974).
8. E. Gibermann, A. Chari-Bitron, S. Millo and S. Gothilf, *Experientia* **30,** 68 (1974).
9. E. Gibermann, S. Gothilf, A. Shahar and T. Bino, *J. Reprod. Fert.* **42,** 389 (1975).
10. T. Bino, A. Chari-Bitron and A. Shahar, *Biochim. biophys. Acta* **288,** 195 (1972).
11. J. M. Mahoney and R. A. Harris, *Biochem. Pharmac.* **21,** 1217 (1972).
12. P. Seeman, M. Chau-Wong and S. Moyyen, *Can. J. Physiol. Pharmac.* **50,** 1193 (1972).
13. D. Bach and I. R. Miller, *J. Membrane Biol.* **11,** 237 (1973).
14. P. Seeman, *Pharmac. Rev.* **24,** 583 (1972).

15. H. Van Zutphen, A. J. Merola, G. P. Brierley and D. G. Cornwell, *Archs Biochem. Biophys.* **152,** 755 (1972).
16. W. D. Seufert, *Nature, Lond.* **207,** 174 (1965).
17. W. D. Seufert, *Biophysics* **10,** 281 (1973).
18. S. G. A. McLaughlin, G. Szabo and G. Eisenman, *J. gen. Physiol.* **58,** 667 (1971).
19. A. Raz and R. Goldman, *Lab. Invest.* **34** (1976).
20. P. E. G. Mann, A. B. Cohen, T. N. Finley and A. J. Ladman, *Lab. Invest.* **25,** 111 (1971).