BBA 75958

THE EFFECTS OF ALCOHOLS ON LIPID BILAYERS: A SPIN LABEL STUDY*

S. J. PATERSON, K. W. BUTLER, P. HUANG**, J. LABELLE, IAN C. P. SMITH AND HENRY SCHNEIDER

Division of Biological Sciences, National Research Council of Canada, Ottawa, KIA OR6 (Canada)

(Received December 30th, 1971)

SUMMARY

Aliphatic alcohols produce changes in the structural arrangement of lipids in bilayers as indicated by studies using the steroid spin probe 3-*spiro*-(2′-(*N*-oxyl-4′,4′-dimethyloxazolidine))cholestane. The concentrations of alcohol corresponding to the onset of perceptible changes in organization correlate well with those causing anesthesia in biological systems, suggesting that anesthesia may be caused by small disruptions of lipid bilayer structure in biological membranes. The data indicate also that lipid bilayer disruption or reorganization is involved in alcohol effects on black lipid membrane conductivity and erythrocyte antihemolysis.

INTRODUCTION

The influence of alcohols on natural and model membranes has been studied extensively in attempts to determine the mechanism by which they act as anesthetics and the nature of the relevant receptor[1]. There is some evidence that the receptor contains hydrophobic binding regions[2], hence that it may be the lipid bilayer component of membranes. The present study of a spin-labelled lipid multibilayer system suggests that anesthesia may be the result of small changes in the organization of lipids in such bilayers.

EXPERIMENTAL

Lipid multibilayers were prepared as described previously[3]. Their thickness when hydrated is about 50 μm. The spin label, 3-*spiro*-(2′-(*N*-oxyl-4′,4′-dimethyloxazolidine)) cholestane, was present at a level of 0.5 mole % of total lipids. Human erythrocyte lipids were extracted[4] from hemoglobin-free ghosts[5]. Beef brain white matter lipids and egg lecithin were isolated as described previously[3,6]. The films were hydrated with 10 mM sodium phosphate buffer containing 0.9 % NaCl at pH 7.0, allowed to equilibrate for 20 min, drained, and the electron spin resonance (ESR) spectrum recorded. In experiments with erythrocyte and brain lipid the cells

* Issued as N.R.C.C. Publication No. 12627.
** N.R.C.C. Summer Student, 1970, 1971.

were then filled with alcohol-containing buffer solution, incubated for 5–10 min, and then a fresh portion of solution was added. This flushing procedure was routinely repeated 3 to 4 times since preliminary experiments indicated that otherwise the desired partitioning was not attained with the higher alcohols. After recording the spectra from an equilibrated film, a higher alcohol concentration was added, and the procedure repeated. With films of egg lecithin containing 15 or 33 mole % cholesterol the flushing procedure was not employed. Instead, the films were incubated in alcohol solutions for 20–30 min and experiments restricted to the lower homologues (up to pentanol); this was necessary because the films tended to erode more easily than those of the other lipids studied. Spectra were recorded with films perpendicular and parallel to the magnetic field using either a Varian E-3 or E-9 ESR spectrometer.

RESULTS AND DISCUSSION

In films of erythrocyte or brain lipids hydrated with the phosphate buffer the cholestane spin label had a high preference for an orientation with its long axis perpendicular to the bilayer plane, as shown by the high degree of spectral anisotropy and the hyperfine splittings of 6 and 19 gauss in the perpendicular and parallel orientations, respectively[6–8]. Fig. 1 illustrates typical spectra obtained with erythrocyte lipid films. It has been suggested that in egg lecithin–cholesterol multibilayers the spin label makes an average angle of about 17° and 10° with the normal, as indicated by the hyperfine splittings of 7.7 ± 0.2 and 6.9 ± 0.2 gauss for 15 and 33 mole % cholesterol, respectively[6]. Because of the rigidity and amphilicity of the spin label, its orientation is taken to reflect closely that of the bilayer lipids, certainly up to a depth equivalent to the length of the steroid nucleus of 9 Å. Detailed discussions of ESR spectra of the cholestane spin label in lipid films, and their interpretation

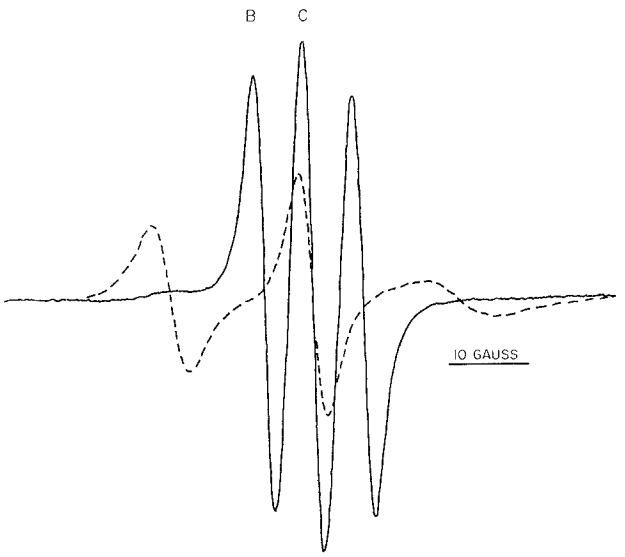

Fig. 1. Typical ESR spectra obtained with human erythrocyte lipid multibilayer films containing the cholestane spin label. Solid and dashed line spectra were obtained with lamellar plane perpendicular and parallel to the spectrometer magnetic field, respectively.

in terms of molecular motion and orientation have been described (refs 6–8; and S. Schreier-Mucillo, D. Marsh, H. Dugas, H. Schneider and I. C. P. Smith, unpublished).

With increasing alcohol concentration a point was reached where the apparent orientation of the spin label in brain and erythrocyte lipid bilayers began to change very rapidly. The hyperfine splittings increased and the ratio of heights of the lines labelled B and C (Fig. 2) decreased, indicating that the proportion of labels oriented normal to the bilayers was decreasing. The changes in B/C were the more obvious and are summarized in Fig. 3. The curves for each alcohol represent an experiment with a single film. Replicate experiments produced similar trends. The difference in B/C at zero or low alcohol concentrations among experiments employing different alcohols results from the inability to prepare films with identical starting B/C ratios. The highest alcohol concentrations employed were determined by the fact that higher concentrations caused removal of the film. Such removal is probably due to a phase change where relatively low molecular weight phospholipid micelles are formed, as indicated by a study of the effects of propanol and lower alcohols on egg lecithin dispersions[9].

The effects of alcohols were completely reversible up to the point where the B/C ratio changed by less than 15 to 25 %; beyond this point only partial reversibility occurred. Reversibility experiments were carried out by repeatedly flushing the films (2 to 6 times) with alcohol-free buffer. Repeated flushing itself had no effect on the B/C ratio as shown in control experiments where films previously untreated with alcohol were flushed with buffer.

Brain white matter lipids behaved like erythrocyte lipids in all respects. However, egg lecithin–cholesterol films eroded rapidly and dissolved off cell surface at alcohol concentrations where the spectral parameters of the other lipid systems began to change rapidly. Since the major structural changes occur at the same alcohol

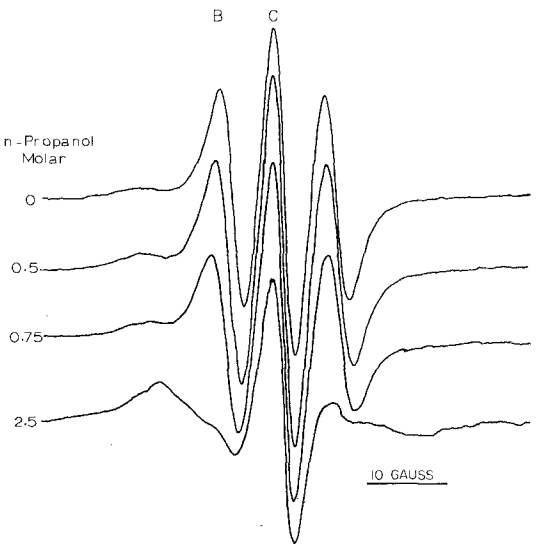

Fig. 2. Effects of *n*-propanol on ESR spectra of human erythrocyte multibilayer films oriented with the lamellar plane perpendicular to the magnetic field. The hyperfine splittings for 0, 0.5, 0.75 and 2.5 M are, respectively, 6.6, 6.9, 6.8 and 7.8 gauss The estimated uncertainty is 0.2 gauss.

concentrations with three diverse lipid systems, the hydrocarbon residues of the lipids, rather than their polar residues, are taken to be responsible for the changes in spin label orientation.

The bilayer disordering data correlate well with the effects of alcohols in anesthetic systems. This is indicated in Fig. 4 which shows the alcohol concentrations which just begin to produce the precipitous decline in B/C and alcohol anesthetic data for tadpoles[10], which is characteristic of such data in general[11]. The agreement suggests that during alcohol anesthesia a slight disorganization of the structural arrangement of membrane lipids in a bilayer array takes place. The data also indicate that disordering of bilayer lipids is involved in alcohol inhibition of erythrocyte hemolysis[12]

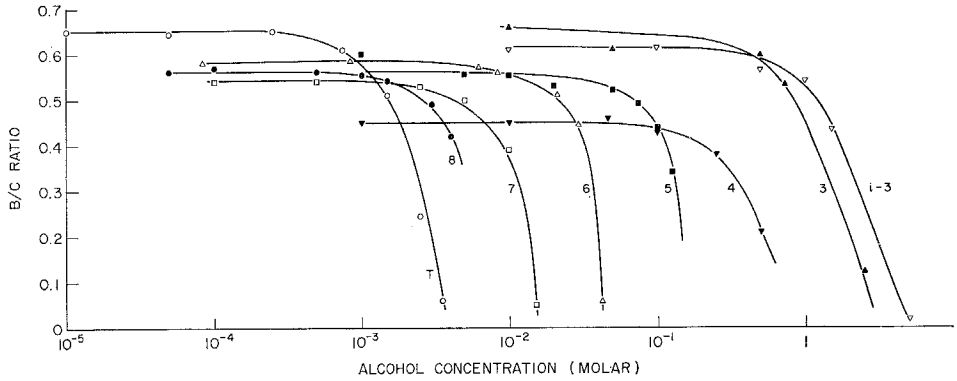

Fig. 3. B/C ratio as a function of alcohol concentration for various alcohols. The numbers refer to the number of carbon atoms in the normal alcohols investigated. Isopropanol is designated as i-3 and thymol as T. The values plotted of B/C for butanol represent one half of those measured.

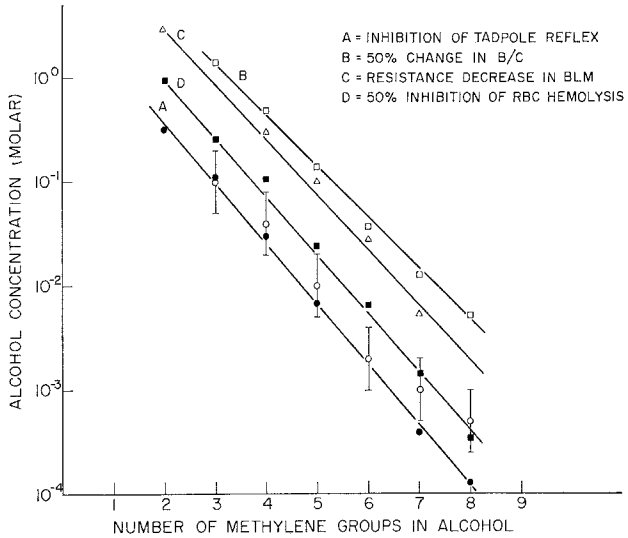

Fig. 4. Alcohol concentrations required to produce particular effects in model anesthetic systems and to cause anesthesia in tadpoles. The open circles are the concentrations where changes in B/C first became evident. The vertical bars denote the uncertainty associated with the determination of these values. Curve B represents the concentrations necessary to produce a 50% change in B/C. RBC = red blood cells; BLM = black lipid membranes.

and the decrease in resistance of black lipid membranes[13], two systems employed as models in anesthetic mechanism studies. The curves for these systems fall between those for a 50% decrease in B/C and the point where B/C just begins to decrease. Thus, a decrease in black lipid membrane resistance requires larger organization changes than that for anesthesia. This behavior also correlates well with the observation that general anesthetics at concentrations found in the blood stream of patients in the surgical stage of anesthesia produce only 8% antihemolysis[14].

Further support for the view that bilayer disorganization is involved in anesthesia is provided by finding that other anesthetics such as thymol (Fig. 4), benzyl alcohol, promethazine and chlorpromazine were found to produce effects on B/C similar to those of alcohols at concentrations where they also produce antihemolysis in erythrocytes.

Anesthesia caused by bilayer disorganization is considered to come about by one, or a combination, of three mechanisms; (i) a change in the properties of membrane bound proteins, (ii) a change in ion-binding properties of proteins or lipids, (iii) a change in ion permeability. Bilayer disorganization implies changes in thickness and inter-lipid distances measured in the lamellar plane. Since critical distances are involved in protein interactions it is expected that the same will be true for protein–lipid interactions. Thus, changes in bilayer dimensional parameters may alter the properties of membrane-bound enzyme or structural protein components, with consequent impairment of membrane function and anesthesia[15]. For ion binding a similar argument holds since membrane function is related to ion binding, and controlling factors such as surface change density, orientation of ionic residues and their mutual separation would all depend on lipid organization. The views regarding the importance of membrane dimensions are consonant with the idea that anesthesia is associated with membrane expansion[14].

The mechanism whereby ion permeability is increased by bilayer disorganization is difficult to specify in detail at present. Some hypotheses relevant to the correlation between disorder, permeability and anesthesia have been described by Johnson and Bangham[16].

CONCLUSION

The organization of lipids in hydrated multibilayers is perturbed by alcohols. The onset of the perturbation with increasing alcohol concentration correlated well with comparable data for their anesthetic effects. Thus, the phenomenon of anesthesia by alcohols may be related, in part, to a disturbance of lipid bilayer structures in biological membranes.

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