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THE EFFECT OF SOME GENERAL ANAESTHETICS ON THE SURFACE POTENTIAL OF LIPID MONOLAYERS

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1 This study sought to investigate the report by Ginsberg (1978) that 0.7 M ethanol brought about a +100 mV change ($\Delta\Delta V$) in the surface potential of glyceryl monooleate (GMO) monolayers formed on KCl, although he predicted that a $\Delta\Delta V$ of −10 mV should have been found.

2 The effect of general anaesthetics such as *n*-alkyl alcohols and pentobarbitone on surface potential (ΔV) and surface tension (γ) of lipid monolayers formed on 145 mM KCl from either glyceryl monooleate (GMO) or phosphatidyl choline (PC) was examined with an Americium-241 air electrode assembly (ΔV) and a platinized platinum dipping plate and force balance (γ).

3 It was found that, as predicted by Ginsberg (1978), addition of 0.7 M ethanol to the aqueous phase bathing either PC or GMO monolayers brings about a negative-going change in interfacial potential ($\Delta\Delta V$).

4 The magnitude of $\Delta\Delta V$ is dependent in a linear fashion on ethanol concentration.

5 Longer chain length alcohols up to *n*-decanol also bring about a negative going change in $\Delta\Delta V$, and the dependence of $\Delta\Delta V$ on anaesthetic activity, with respect to increasing chain length of anaesthetic, is consistent with Traube's law.

6 Pentobarbitone added to the aqueous phase bathing the monolayer also elicits a negative $\Delta\Delta V$, a finding which rules out the possibility of adsorption of the volatile alcohols to the measuring electrode.

7 The findings are discussed in terms of the proposition that increasing disorder in an array of fixed dipoles, such as might occur in a bilayer exposed to anaesthetic, would result in a lowering of the electrostatic barrier to the predominantly impermeable cation.

Introduction

It is well accepted that a number of lipid-soluble compounds which behave as general anaesthetics do so through their interaction with membranes, but the precise site and nature of their action is not known. A general mechanism by which anaesthetics might exert an effect is by altering the ionic permeability, and therefore electrical conductance, of cell membranes (Bangham, Standish & Miller, 1965). Among a number of theories which provide an explanation consistent with such a mode of action is one in which the membrane ionic permeability is controlled by the sign and magnitude of the membrane surface charge (Bangham, Standish & Watkins, 1965; Chandler, Hodgkin & Meves, 1965; McLaughlin, Szabo, Eisenman & Ciani, 1970). Later, Liberman & Topaly (1969) and Le Blanc (1970) considered the possible contribution of a dipole array associated with the orientated membrane molecules in accounting for the differential permeability to nearly similar anions and cations of membranes without obvious surface charge, e.g. phosphatidyl choline (PC). Both sources of electrical potential were discussed by Haydon & Hladky (1972) in relation to the control of membrane conductance, and some measurements made by Haydon & Myers (1973). The conclusion was that ion conductivity and selectivity is determined by the total potential profile across the membrane interface.

In a recent paper, Ginsberg (1978) attempted to correlate the effect of ethanol on artificial lipid bilayer conductance with possible changes occurring in the surface potential ($\Delta\Delta V$) of the bilayer material, glyceryl monooleate (GMO), spread as a monolayer. Ginsberg reported that the effect of 0.7 M ethanol on GMO bilayers was to increase the specific membrane conductance by approximately one-third. It was suggested that were this conductance change due to an alteration in membrane surface potential, the magnitude of such an alteration could be calculated using an expression derived from Hladky & Haydon (1973), and would amount to a decrement of 10 mV, the aqueous potential being shifted in the *negative* direction. However, Ginsberg's subsequent measurements, using the vibrating gold plate air electrode, indicated that the surface potential of monolayers formed from

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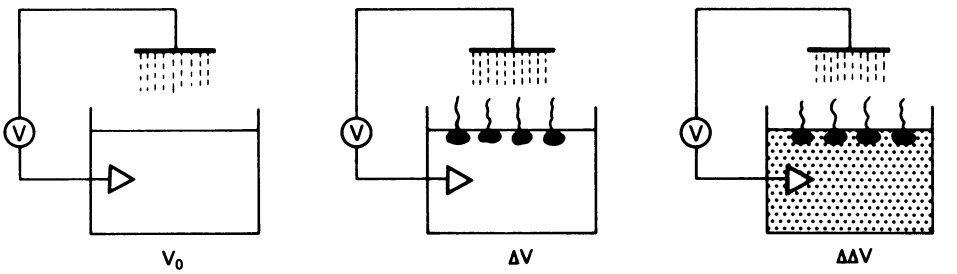

Figure 1 Diagrammatic representation of the experimental measurement of V_0, ΔV and $\Delta\Delta V$. V_0 is the initial value of potential measured for a clean surface, being the potential measured by the electrometer between a KCl saturated calomel reference electrode and the ionisable Americium-241 air electrode. ΔV is used here to denote the change in potential when molecules of either phosphatidylcholine (PC) or glyceryl monooleate (GMO) lipids are added at the air water interface. $\Delta\Delta V$ denotes the further change in surface potential brought about when the surface-active anaesthetic molecules are added to the aqueous phase beneath the monolayer.

GMO shifted more than 100 mV in the *positive* direction. Finally, it was pointed out that precise estimation of this potential increase was difficult, presumably due to volatility of the ethanol.

The present paper describes observations made both on GMO and PC and our results suggest that Ginsberg's predicted value (i.e. a −10 mV change upon addition of 0.7 M ethanol) is more precisely the value obtained experimentally. It is suggested on the basis of the present results with ethanol and longer chain length alcohols of a less volatile nature, that the discrepancy in the results may arise from a combination of measuring technique (i.e. vibrating gold plate air electrode used by Ginsberg and an Americium air electrode used here) and volatility of the ethanol leading to possible interaction with the gold plate air electrode.

A further but trivial explanation for the noted discrepancy might be that Ginsberg's measurements were made on monolayers of less than full compression, particularly those with large values at area per molecule. However, Ginsberg (personal communication) has since noted that the monolayers of GMO in hexadecane used in his measurements contained detectable lenses of the lipid mixture, and thus were probably fully compressed. It is thus unlikely that such an explanation is valid.

The present measurements have been extended to an examination of the effect of ethanol and higher alcohols on surface potential and surface tension (γ) at a variety of areas per molecule for both lipids. These observations suggest that in the case of close packed lipid monolayers, there is a negative-going change in surface potential which is dependent on and linear with respect to concentration of the anaesthetic. As well, in the case of expanded monolayers, the experiments demonstrate that the alcohols themselves contribute a positive-going component to surface potential, the magnitude of which is dependent on the initial surface concentration of lipid used to form the monolayer and the aqueous concentration of the alcohol.

Methods

Monolayer measurements

Measurements of monolayer surface potential alone were made in a teflon trough which had a surface area of 3.15 × 3.75 cm and a volume of approximately 10 ml. The rear of the trough had two small apertures, one through which additions of anaesthetic could be made beneath the surface of the monolayer and one through which a calomel reference electrode was inserted. A small glass-enclosed stirring bar rested on the bottom of the trough and was rotated in the trough by an externally placed, motor-driven magnet. An Americium-241 air electrode was positioned above the trough and connected to the high impedence input of a Vibron electrometer. The calomel reference electrode was connected to the earthed low impedance input of the Vibron. The apparatus was electrostatically shielded by a brass box which surrounded the trough and electrodes. For a definition of the terms V_0, ΔV and $\Delta\Delta V$ see Figure 1.

The trough was cleaned by washing first in methanolic potassium hydroxide followed by rinsing in water and a further wash in concentrated nitric acid. This was followed by extensive washing in distilled water. It was found that when the electrolyte surface was entirely clean, an initial potential (V_0) for the circuit with no monolayer present was −415 to −420 mV negative, with respect to the aqueous phase. To obtain these values, it was frequently necessary to

repeat the washing procedure until a potential of −415 to −420 mV was measured.

The electrolyte and concentration used throughout these experiments was 0.145 M KCl. In all experiments 10 ml of electrolyte solution was added to the trough by means of a glass syringe. Solutions of both GMO and PC were prepared as 0.5 mM solutions in petroleum ether and were applied to the surface of the electrolyte by means of a microlitre syringe with micrometer head. The ΔV observed upon addition of the lipid solution was positive-going and usually became stable after about 30 s. More or less lipid was added to the surface of the electrolyte depending on whether it was desired to study an expanded or a close packed monolayer.

In order to measure the surface coverage accurately, i.e. area per molecule of the lipid under examination, parallel measurements of surface potential (ΔV) and surface tension (γ) were also made on a large trough measuring 20.9 × 36.2 cm with a capacity of about 2 litres. Areas per molecule of the lipid were calculated by dividing the area on which they were confined by the number of molecules. Surface area was varied by moving a Teflon barrier across the trough surface. Surface tension (γ) was measured by recording the downward pull on a platinized platinum plate of 2.08 cm width, with a CI force balance calibrated in mN/m. Surface potential was monitored by positioning a screened Americium-241 electrode within 0.5 cm of the surface, and connected as before to the Vibron electrometer.

Preparation of solutions

Where it was desired to study the effect of higher alcohols on membrane surface tension and surface potential, solutions of the KCl electrolyte were saturated for 48 h with bulk quantities of the alcohols (ethanol to decanol). The saturation process was accomplished in separatory funnels which were gently agitated over this period. Stated activities (C/C_o) are meant to imply the percentage saturation of the aqueous phase with alcohol, i.e. an activity of 0.1 indicates 1.0 ml alcohol-saturated KCl solution plus 9.0 ml of alcohol-free KCl solution.

The pH of all solutions was approximately 5.6, and the temperature throughout the experiments was 18° to 21°C.

Materials

GMO was obtained as monoolein from Sigma Chemical Company. PC was extracted from egg yolks and purified by silicic acid chromatography. Hydrocarbon solvents were obtained in pure form from Fisons & Koch Light. Water was twice distilled, the final distillation being from potassium permanganate.

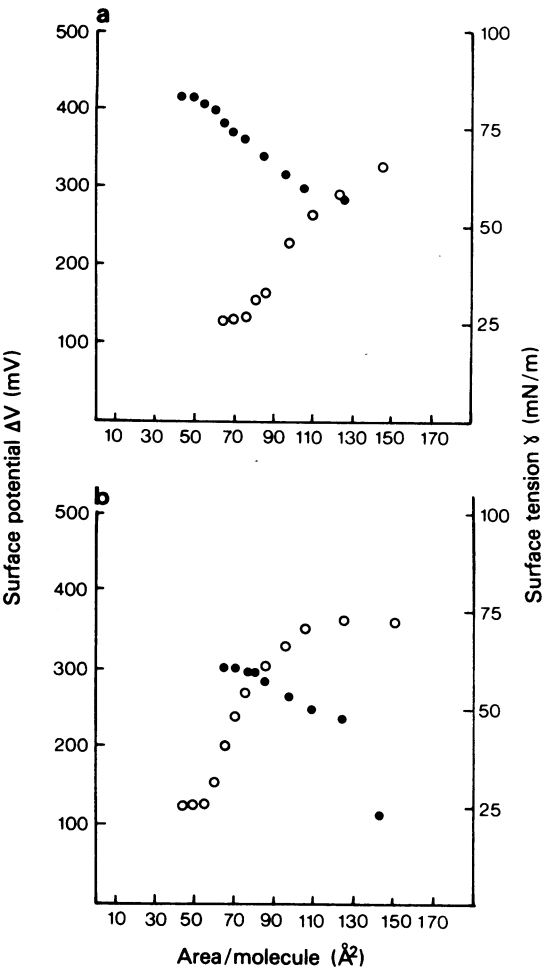

Figure 2 (a) The relationship between surface potential (●) surface tension (○) and area per molecule for phosphatidyl choline monolayers formed on 145 mM KCl. (b) The relationship between surface potential (●), surface tension (○) and area per molecule for glycero-monooleate monolayers formed on 145 mM KCl.

Pentobarbitone was obtained from May & Baker Ltd., in the acid form. It was dissolved in 0.145 M KCl, and solubilized by addition of KOH.

Results and Discussion

Figure 2 shows the dependence of surface potential (ΔV) and surface tension (γ) on the area per molecule for lipid monolayers formed from either GMO or PC. The values were derived from simultaneous measurements made with the large trough described in the Methods section.

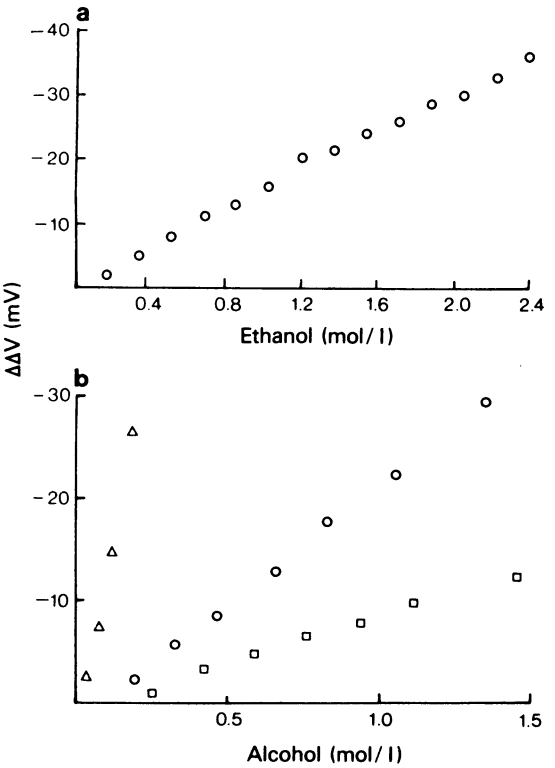

Figure 3 (a) The dependence of ΔΔV on ethanol concentration for a fully compressed phosphatidyl choline (PC) monolayer formed on 145 mM KCl. The initial ΔV recorded upon monolayer formation was +416 mV at a calculated surface packing density of 1 PC molecule per 30 Å^2. Note the negative going surface potential obtained with increasing ethanol addition to the aqueous phase beneath the monolayer. (b) The effect of ethanol (□), propanol (○) and butanol (△) on the ΔΔV of monolayers formed from glyceryl monooleate on 145 mM KCl. Note the increasing slope of potential versus alcohol concentration for increasing chain length of alcohol used.

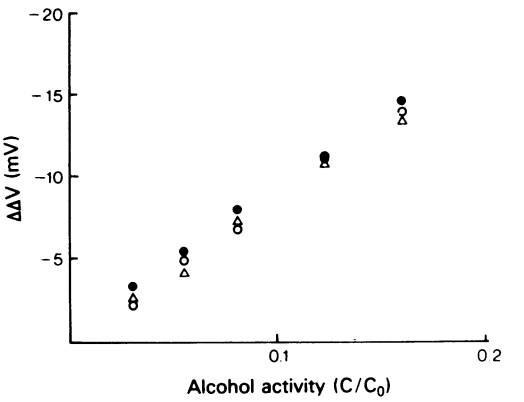

Figure 4 The dependence of ΔΔV on alcohol activity for ethanol, propanol and butanol in 145 mM KCl beneath fully compressed monolayers of phosphatidyl choline. The use of activity to express a fraction of saturation for the alcohols is explained in the text. Note the similar slopes of ΔΔV versus alcohol activity measured for comparable activities of the varying chain length alcohols, showing that Traube's law is obeyed for the present experimental system. (○) Ethanol, (●) propanol, (△) butanol.

It can be seen (Figure 2a) for GMO monolayers formed on 145 mM KCl that the maximum value of ΔV was approximately +310 mV at minimum area per molecule. For PC monolayers (Figure 2b), under similar conditions, a maximum value of ΔV of +420 mV was measured at minimum area per molecule.

Values of ΔV for the monolayers of highest packing density corresponded well with those obtained when excess lipid was placed on the surface, i.e. equilibrium lipid tensions were similar in both large and small troughs.

In contrast to Ginsberg's (1978) results, we observed a progressive negative-going decrement of the ΔV (ΔΔV) of both PC and GMO monolayers at their equilibrium spreading pressures (π_e), as the substrate ethanol concentration was raised (Figure 3a). Furthermore, propanol was more effective than ethanol in effecting a similar decrement in ΔΔV, and butanol was more effective than propanol, in accordance with Traube's rule (Figure 3b).

As expected, the ΔΔV was of the same sign and magnitude when fractional concentrations (i.e. similar activities) of saturated solutions of the alcohols ethanol, propanol and butanol were tested (Figure 4). This result with the longer chain alcohols eliminated possible doubt as to whether the observed change in surface potential (ΔΔV) could have been due to adsorption of the volatile alcohols on to the Americium electrode.

To investigate whether the decrement in ΔΔV brought about by alcohols is a more generalized property of general anaesthetics, a range of concentrations of a non-volatile anaesthetic, Na pentobarbitone were tested. Figure 5 shows that pentobarbitone also brings about a negative ΔΔV at the stated concentrations. This result also rules out the possibility that adsorption of the anaesthetic molecules on to the electrode surface could account for the observed surface potential changes (ΔΔV) brought about by the alcohols. It was similarly found that chloroform, at a concentration of 6×10^{-3} M brings about a 10 mV decrement in ΔV in both PC and GMO.

It should be noted that the ΔΔV for a given concentration of ethanol was somewhat greater for PC than

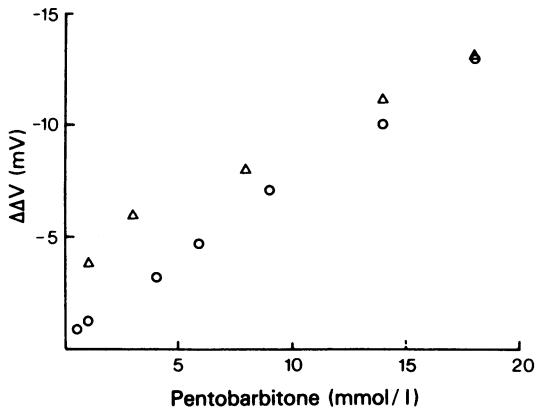

Figure 5 The effect of sodium pentobarbitone on the ΔΔV of fully compressed phosphatidyl choline (PC, ○) and glyceryl monooleate (GMO, △) monolayers formed on 145 mM KCl.

GMO; nevertheless, the sign and magnitude of the changes (ΔΔV) were compatible with those required by Ginsberg.

Dependence of ΔΔV on area per molecule

As a separate part of this investigation the effect of ethanol on expanded PC and GMO monolayers was also examined. To do this, different ethanol concentrations were established in the aqueous phase prior to formation of the monolayer, and various areas per molecules for these monolayers were examined by successive additions of lipid to the surface from a petroleum ether solution.

It was found that additions of ethanol to the aqueous solution in the absence of a monolayer brought about, as expected, a large change in the ΔV. This change was dependent on ethanol concentration, as seen in Figure 6. It will be apparent from Figure 6 that the addition of ethanol to either PC or GMO monolayers at large areas per molecule can result in large positive-going changes in ΔΔV, but as the surface density of PC or GMO increases, so the ΔΔV attributable to the alcohol diminishes, until as already shown, it becomes negative (Figure 8).

Experimentally, the complementary contribution to the ΔV of the lipid and alcohol may be further observed by forming expanded monolayers with GMO (as in Figure 7) or PC, such that varying initial values of ΔV are obtained, subsequently making an ethanol addition at a given concentration. When this is done with 0.7 M ethanol added beneath GMO monolayers, the magnitude of the ethanol-induced ΔΔV is related to the ΔV already generated by GMO addition. The magnitude of the ethanol-induced ΔΔV becomes smaller with increasing packing density such that at high area per molecule the ethanol-induced

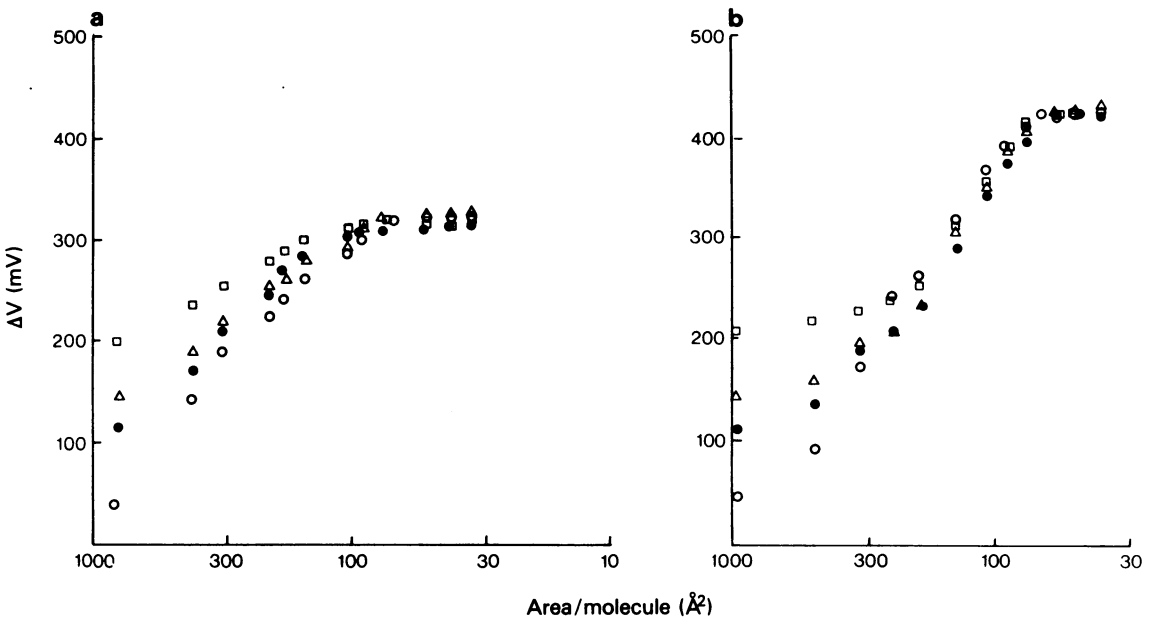

Figure 6 The effect of ethanol on ΔV measured at varying areas per molecule for glyceryl monooleate (a) and phosphatidyl choline (b) monolayers formed on 145 mM KCl. (○) No ethanol present; (●) 0.175 M ethanol; (□) 0.35 M ethanol; (△) 0.7 M ethanol.

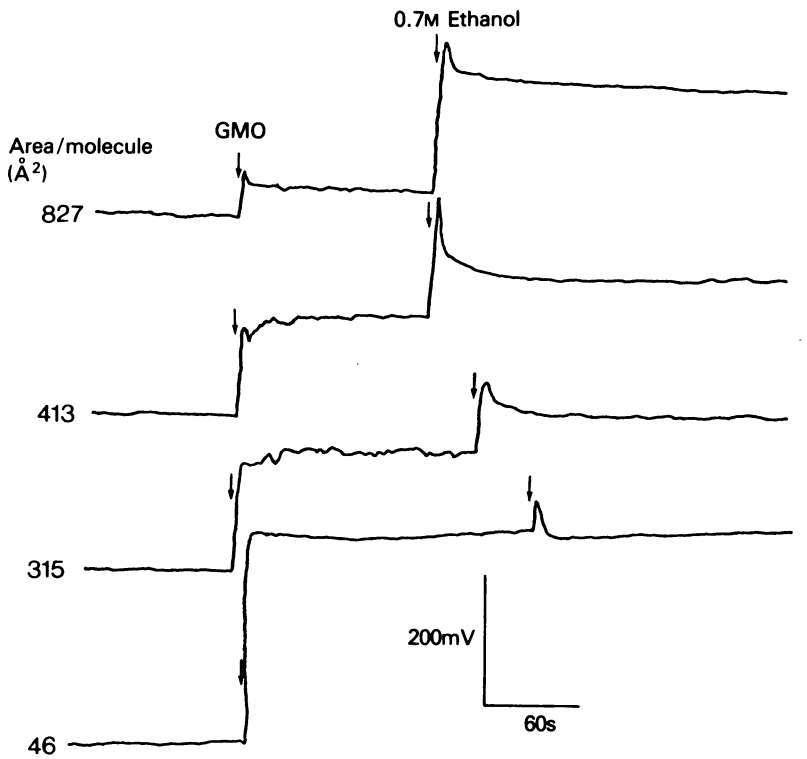

Figure 7 Experimental records showing the effect of lipid and ethanol addition on the surface potential of glyceryl monooleate (GMO) monolayers formed on 145 mM KCl. GMO was added in petroleum ether at the air/water interface where shown, at a concentration to yield the areas/molecule given to the left of the figure. Where noted, ethanol was added to the aqueous phase at a concentration of 0.7 M. It can be seen that at large values of area per molecule, ethanol addition elicits a large $\Delta\Delta V$, which in turn decreases in magnitude with decreasing values of area per molecule until it reverses sign for the case of a fully compressed monolayer (lower record). Magnitude of surface potential changes and their time course are given by the bracket in lower right-hand corner.

$\Delta\Delta V$ is very large, as much as 200 mV or more, and at low area per molecule, or high packing density, the ethanol-induced increase in $\Delta\Delta V$ is reduced. For a fully compressed monolayer (lower trace in Figure 6) the $\Delta\Delta V$ is negative-going as previously described. Similar results are observed for PC monolayers.

These results are summarised in Figure 8, for a representative group of experiments on PC monolayers. It can be seen that, at large areas per molecule, the $\Delta\Delta V$ upon ethanol addition is positive-going, whereas at smaller areas per molecule, the value of $\Delta\Delta V$ becomes negative.

Conclusion

Monolayer studies are not obviously relevant to an understanding of how ions cross model or real cell membranes, but they do represent a valid method of measuring the overall electrostatic potential energy to the centre of a bilayer up and over which an ion might be expected to pass. If the permeability of membranes is modified, in this instance by the addition of anaesthetics, then it is significant that all the substances used in this study, including the ionisable compound pentobarbitone, bring about a similar change in surface potential ($\Delta\Delta V$) both in sign and magnitude at concentrations which bring about equivalent biological activity.

For instance, the concentration of butanol required to bring about a 50% reduction in nervous conductance in the frog sciatic nerve is 6.8×10^{-2} mol/litre (Seeman, 1972). Such a concentration would bring about a $\Delta\Delta V$ of -4 mV in the present monolayer experiments. However, it is important to note that in these experiments examining anaesthetic effects on surface potentials the concentrations required to elicit an effect exceed that required for general anaesthesia and, indeed, are in a concentration range which

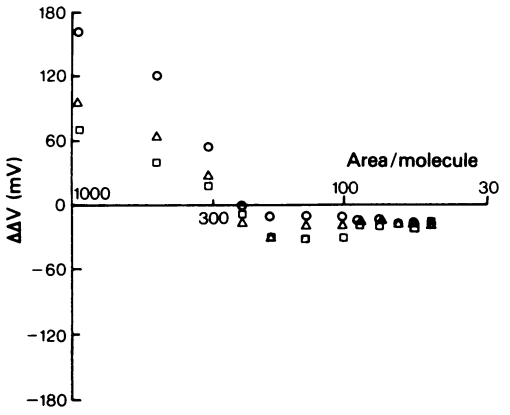

Figure 8 Compiled data showing the ΔΔV upon ethanol addition for varying values of area per molecule for phosphatidyl choline (PC). The ΔΔV values in this figure are calculated as the difference in surface potential between a monolayer on pure 145 mM KCl and one containing either 0.175 M ethanol (□), 0.35 M ethanol, (△) and 0.7 M ethanol (○).

exceeds that necessary to block nervous conduction. Nevertheless, the results are suggestive of a graded and linear response of surface potential to anaesthetic concentration, and a response which extrapolates to zero.

From earlier studies with liposomes, it has been argued that anaesthetics change the Gibbs free energy of the membrane phase (Hill, 1974), in the sense that as anaesthetics dissolve in the membrane or as the membrane is warmed up, there follows a volume increment associated with a disordering process and a lowering of an energy of activation for some membrane constraint. Our results support Ginsberg's proposition that a consequence of increasing disorder in an array of fixed dipoles, such as might exist in a bilayer, would result in a lowering of the electrostatic barrier to cations. Whether these relatively small changes in conductance or permeability are directly relevant to the anaesthetized state remains to be determined.

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