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Surface potential changes in lipid monolayers and the 'cut-off' in anaesthetic effects of *N*-alkanols

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The effects of 0.09 saturated solutions of the *n*-alkanols *n*-hexanol to *n*-tridecanol on the surface (compensation) potential of lipid monolayers have been examined. Actions on monolayers spread from pure egg phosphatidylcholine have been compared with effects on a system containing a 2:1 mole ratio of egg phosphatidylcholine and cholesterol. The mean compensation potential for the pure phospholipid system was 475 ± 9 mV; addition of cholesterol increased the potential to 503 ± 10 mV. All *n*-alkanols tested reduced the surface potential in both systems. The reduction was larger in the pure phospholipid system but the difference in effect between lipid systems declined as the *n*-alkanol chainlength increased, becoming negligible by *n*-tridecanol. These results are considered in relation to the 'cut-off' in biological activity of *n*-alkanols around *n*-tridecanol.

It has long been recognised that the *n*-alkanols become progressively less active as both local and general anaesthetics beyond *n*-dodecanol and that *n*-tetradecanol is inactive [1–4]. For general anaesthesia at least, this 'cut-off' has been ascribed to a failure of the larger molecules to adsorb to the site of anaesthetic action, be it predominantly lipid [2,5] or protein [6] in nature. However, the available data concerning the adsorption of long chain alkanols and other amphipathic compounds to lipid structures do not entirely support the view of a cut-off in the region C_{13-14}. Thus the effects of *n*-alkanols on gramicidin channels in planar lipid bilayers [7,8] and on the surface tension of lipid bilayers [8] are maintained up to *n*-hexadecanol at least and a recent study of the adsorption of *n*-alkyltrimethylammonium ions to liposomes found continued adsorption up to C_{18} [9].

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We have now systematically investigated the effects of the *n*-alkanol homologous series between *n*-hexanol and *n*-tridecanol on the surface potential of lipid monolayers spread at an air/0.5 M NaCl solution interface. It has been suggested previously that alterations in the lipid dipole potential of excitable membranes could be involved in local [10–12] and general anaesthesia [13]. The differential effects of *n*-alkanols on the surface potentials of monolayers formed with and without cholesterol, described below, suggest one possible mechanism for a cut-off in local anaesthetic activity.

Measurements of lipid monolayer surface (compensation) potentials were made by the vibrating-plate technique, essentially as described by Haydon and Myers [14]. The procedure involved balancing the experimental potential against a variable voltage applied from a potentiometer. Readings were taken at one to two minute intervals throughout the experiment. After obtaining a steady value for the potential at an air/saline

interface sufficient lipid was added, dispersed in petroleum ether, to form a monolayer plus lenses of excess lipid. The compensation potential changed on addition of the lipid and reached a new steady value (ΔV) after 2–5 min (evaporation of the solvent presumably accounts for most of the delay in achieving a steady state). The alcohol was added once a satisfactory reading for the lipid surface potential had been obtained. The experimental dish initially contained 100 ml of 0.5 M NaCl and 10 ml of a saturated solution of the required alkanol was added via a syringe. The solution was then stirred. This procedure involved some manipulation of the vibrating plate and, of course, the monolayer was mechanically disturbed. A new reading of the compensation potential was then made and any permanent change ($\Delta\Delta V$) ascribed to the addition of *n*-alkanol. Checks were made to ensure that simple manipulation of the plate or addition of more saline did not cause a significant change in potential.

Egg phosphatidylcholine was obtained from Lipid Products Ltd. (Redhill, U.K.) and the cholesterol from Sigma (Poole, U.K.). The *n*-alkanols used were all puriss or purum grade and supplied by BDH (Poole, U.K.). The NaCl was AnalaR grade and had been roasted overnight at 700°C to remove organic impurities. Water used was double-distilled, the second time from a quartz still. The dish, syringes, stirrer bars, glass parts of the bath electrode and any volumetric glassware used were vigorously cleaned with chromic acid. Reproducible and steady values for the lipid surface potentials were only obtained after strict attention to the cleaning procedure. The saline solution was unbuffered (the electrophoretic mobility, and hence zeta potential, of phosphatidylcholine vesicles is insensitive to changes in pH over the range 2–11 (see ref. 9)) and the experiments were carried out at room temperature (19–23°C).

Fig. 1 shows the measurements made during three experiments using *n*-hexanol and three *n*-heptanol. The abrupt decrease in potential following the addition of alkanol is clearly shown, as is the lack of effect of further addition of lipid. Table I gives the values obtained for the surface potentials of egg phosphatidylcholine (egg PC) monolayers with and without cholesterol. The average value of 475 ± 9 mV for the pure phos-

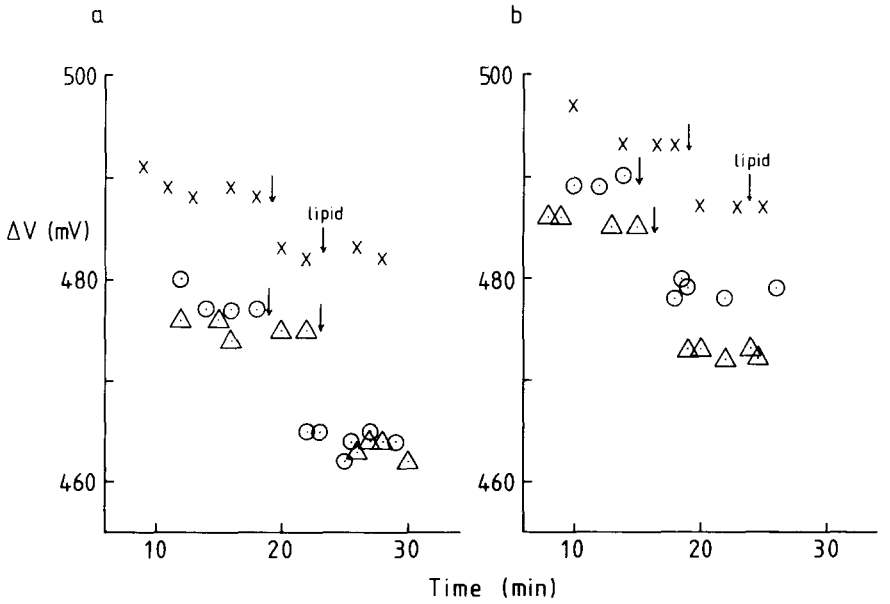

Fig. 1. (a) The effect of addition of *n*-hexanol at a final fractional saturation of 0.09 on the surface potential (ΔV) of lipid monolayers on 0.5 M NaCl. Unmarked arrows denote the addition of *n*-alkanol solution. Arrows marked 'lipid' denote the addition of further quantities of lipid solution. Zero time is taken as the first reading of a potential at the air/saline interface. △ and ○, egg PC monolayers; ×, egg PC/cholesterol (2:1 mole ratio) monolayers. (b) As for (a) but for *n*-heptanol.

TABLE I

The surface potential (ΔV) of various monolayers at the air/0.5 M NaCl solution interface

System	Surface potential (mV) (mean ± S.D.)	Number of experiments
Egg PC	475 ± 9	62
Egg PC/cholesterol (2:1 mole ratio)	503 ± 10	49
Egg PC/hexadecanol (2:1 mole ratio)	475 ± 2 (range)	3

pholipid monolayer may be compared with 447 mV reported by Hladky and Haydon [15] for egg PC/decane monolayers and 420 and 440 mV reported by Bangham and Mason (petroleum ether) [13] and Reyes et al. [16] (*n*-pentane), respectively. Given standard deviations of approx. 10 mV and the expected biological variability in the material this is reasonable agreement. Addition of cholesterol (2 : 1 mole ratio phospholipid to cholesterol) increased the mean compensation potential to 503 ± 10 mV. Such an increase would not be predicted by a simple composition-weighted averaging of individual surface potentials since pure cholesterol monolayers give potentials of approx. 390 mV (see Ref. 10). However, this relatively small change is consistent with the report by Benz and Cros [23] of a lack of significant effect of cholesterol on transport of lipophilic anions through dioleoylphosphatidylcholine bilayers.

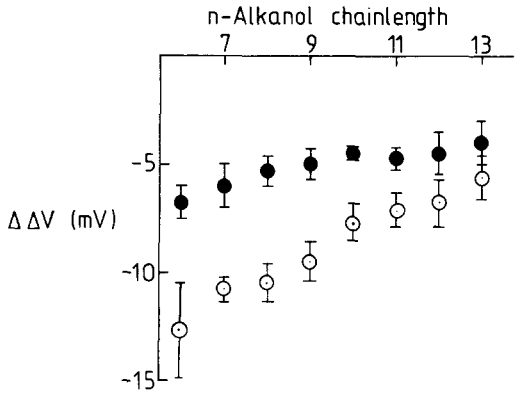

Fig. 2. The dependence of the change in lipid monolayer surface potential ($\Delta\Delta V$) on *n*-alkanol chainlength. All alkanols were at a fractional saturation of 0.09. O, pure egg PC monolayers; ●, 2:1 mole ratio egg PC/cholesterol. Each point is the mean of three or four readings. Error bars give respectively the range of values or the standard error of the mean.

Fig. 2 shows the change in dipole potential ($\Delta\Delta V$) as a function of *n*-alkanol chainlength (*n*-hexanol to *n*-tridecanol) for the egg PC and egg PC/cholesterol systems. The choice of alkanols was governed by the following considerations. First, for volatile alkanols, adsorption of the alkanol to the vibrating plate is a possible source of error. Accordingly the more volatile lower chainlength compounds were not studied in detail, although *n*-pentanol was shown to act similarly to *n*-hexanol. The direction of a stream of air at the plate during measurement of the action of lower alkanols had no observable effect on the result, indicating that volatility was not a major problem in the systems studied. Second, for the longer chainlength compounds the method of adding the alkanol to the bath could give rise to problems of depletion for alkanols much larger than *n*-tridecanol. It was felt that an alternative method using a continual flow of alkanol solution could produce unacceptable disturbance of the monolayer. However, Table I shows the measured surface potential of monolayers spread from a 2 : 1 mole ratio mixture of egg PC and *n*-hexadecanol, where delivery of the alkanol is not via the aqueous phase and therefore where depletion could not be a problem. These monolayers gave values indistinguishable from the control egg PC system, supporting the trend apparent in Fig. 2. Two more egg PC/*n*-hexadecanol mole ratios (4:1 and 10:1) were tested, with the same result.

Fig. 2 shows that all the *n*-alkanols studied reduced the monolayer surface potential in both the egg PC and egg PC/cholesterol system. However, while that reduction was always larger for the pure egg PC monolayer, the difference in effect clearly declined with increasing chain length, so that by *n*-tridecanol it was no longer significant. In these systems all the lipids were either neutral or zwitterionic and the aqueous phase was 0.5 M NaCl. Under these conditions the contribution to the surface potential of ionic double layers is likely to have been very small (≤ 1 mV) [17,18] and the observed changes to have arisen from the reorganisation of the intramolecular surface dipoles. The decline in surface potential difference illustrated in Fig. 2 could account for the cut-off in local anaesthesia by *n*-alkanols re-

ported to occur in both myelinated [3] and unmyelinated [4,19] nerves. This possibility is based on the suggestion [12,20,21] that in general terms the activity of a voltage-gated membrane ion channel could be altered by a drug induced reduction in dipole potential, provided that reduction was not equal at either side of the membrane. Such an asymmetrical alteration in potential would alter the membrane electrical field, while a symmetrical reduction would leave the field unchanged. If the ion channel responds to that component of the membrane field which includes the dipole potential, rather than to the potential difference across the bulk aqueous phases, its activity should be affected. Reduced to its simplest level, this argument suggests that if one side of a membrane contained pure egg PC and the other a 2 : 1 mixture of egg PC and cholesterol, the results of Fig. 2 indicate that treatment with 0.09 saturated *n*-hexanol would change the overall membrane potential by 5 to 6 mV, while *n*-tridecanol would induce a change of only approx. 1 mV. Consistent with this, preliminary studies on the Na^+ current of the squid giant axon (ref. 19, and Elliott, J.R. and Haydon, D.A., unpublished observations) show that the shift in the voltage dependence of Na^+ current activation caused by 0.1 saturated solutions of *n*-alkanols declines from about 11 mV for *n*-hexanol to about 6 mV for *n*-nonanol. Thus, an asymmetric effect of adsorption of the *n*-alkanols in the nerve membrane, owing perhaps to differing cholesterol levels on the two sides [22] could account for the cut-off in local anaesthesia.

It is less obvious that changes in membrane surface potential per se would influence the activity of a non-voltage-gated ion channel or would be relevant to the cut-off observed in general anaesthesia. However, the effects on surface potential must derive from some perturbation in membrane structure and this perturbation may itself affect channel function. Given the present difficulty in understanding quantitatively the origins of the dipole potential, speculation as to the actual mechanism of alkanol, or cholesterol, action is unlikely to be profitable.

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