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A quantitative explanation of the effects of some alcohols on gramicidin single-channel lifetime

J.R. Elliott, D. Needham *, J.P. Dilger **, O. Brandt *** and D.A. Haydon

Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG (U.K.)

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The effects of *n*-decanol, *n*-hexadecanol, *n*-octyl(oxyethylene)$_3$ alcohol and cholesterol on gramicidin single-channel lifetime in planar lipid bilayers have been determined. The bilayers used were formed from a solution of monoolein in squalene. Measurements have also been made of the above compounds' effects on membrane thickness (as measured by electrical capacity and optical reflectance technique) and surface tension (as derived from bulk interfacial tension and bilayer-lens contact angle measurements). The reduction in single-channel lifetime caused by the *n*-alkanols may be accounted for quantitatively in terms of the effects of these compounds on bilayer thickness and surface tension. The *n*-octyl(oxyethylene)$_3$ alcohol caused an increase in single-channel lifetime which is also consistent with the thickness/tension theory. The reduction in channel lifetime caused by cholesterol, however, was much larger than would be predicted from its effects on bilayer thickness and surface tension.

The lifetimes of gramicidin channels in planar lipid bilayers formed from various lipid/hydrocarbon mixtures have been shown to depend on the thickness and surface tension of the bilayer and a quantitative theory has been advanced to explain these effects [1].

It was proposed that the work done by the bilayer tension contributes to the free energy of formation of the transition state between the conducting dimer and the non-conducting monomer species and that the angle of action, and therefore effectiveness, of that tension depends on the thickness difference between the bilayer and the dimer. The hydrophobic length of the conducting dimer was estimated to be around 2.2 nm.

The *n*-alkanols increase the surface tension of planar bilayers [2,3], have a slight effect on bilayer thickness [2,4] and decrease the lifetime of gramicidin channels in monoolein/squalane bilayers [5]. Cholesterol has also been shown to decrease channel lifetime and was far more potent than any of the *n*-alkanols used [5]. It was suggested that surface tension increases were important in the effects of alcohols on channel lifetime but no quantitative analysis was carried out. We have now measured the effects of two *n*-alkanols, *n*-decanol and *n*-hexadecanol, cholesterol and the detergent *n*-octyl(oxyethylene)$_3$ alcohol on bilayer thickness and surface tension and on gramicidin channel lifetime in monoolein/squalene bilayers. The results have been analysed using the equations proposed in Ref. 1 and the success of an explanation of the various effects on

* Present address: Department of Pathology, University of British Columbia, 2211 Wesbrook Mall, Vancouver, British Columbia V6T 1W5, Canada.
** Present address: Department of Physiology, University of Medicine and Dentistry of New Jersey, 100 Bergen Street, Newark, NJ 07103, U.S.A.
*** Present address: Department of Physics, St. Lawrence University, Canton, NY 13617, U.S.A.

channel lifetime based solely on bilayer thickness and surface tension assessed.

The bilayers were formed and electrical capacity per unit area measurements made as described previously [6]. Capacities were measured at 500 Hz and the thickness, h, of the bilayer chain region calculated from the expression

$$h = \frac{\varepsilon_0 \varepsilon}{C} \quad (1)$$

where ε_0 is the permittivity of free space, ε is the dielectric constant of the chain region (taken to be 2.20) and C is the capacity per unit area of the bilayer. Gramicidin single-channel lifetimes were measured as described by Hladky and Haydon [7] and total bilayer thickness calculated from measurements of optical reflectance as described by Dilger [8]. Bulk interfacial tensions and bilayer-lens contact angles were measured by the method of Needham and Haydon [9] and the half-bilayer tension calculated as the product of the bulk tension and the cosine of the contact angle.

Bilayers were formed from solutions of 28 mM monoolein in squalene. *n*-Decanol, *n*-hexadecanol and cholesterol concentrations (Table I) are those in the bulk oil phase. *n*-Octyl(oxyethylene)$_3$ alcohol was dissolved in the aqueous phase and the concentration given refers to that phase. Oil and aqueous phases were left in contact to equilibrate before each experiment. This was found to be of particular importance in avoiding time effects in the measurement of bilayer surface tension.

Table I gives, for each system investigated: the bilayer electrical capacity per unit area and the hydrocarbon region thickness derived from that capacity; the total bilayer thickness derived from optical reflectance measurements; the bulk interfacial tension and bilayer-lens contact angle and the half bilayer tension ($\sigma/2$) derived from the two, and the mean gramicidin channel lifetime (τ) for a given number of channels. The experimental conditions for each measurement are also given. The bilayer-lens contact angle was not measured for the *n*-octyl(oxyethylene)$_3$ alcohol system and the control value has been used to calculate $\sigma/2$. This may be justified because altering the assumed con-

TABLE I

THE EFFECTS OF VARIOUS ALCOHOLS ON BILAYER THICKNESS AND SURFACE TENSION AND ON GRAMICIDIN CHANNEL LIFETIME IN MONOOLEIN/SQUALENE BILAYERS

The applied potential for channel lifetime measurements was ±50 or ±100 mV. The aqueous solution was 0.1 M NaCl or 0.5 M KCl (except for reflectance measurements where a range of concentrations must be used [8]). $T = 23°C$ for channel lifetime and bilayer thickness measurements, 24.5°C for bilayer tension measurements. Experimental values are given as mean ± S.D.

System	Capacity per unit area ($nF \cdot mm^{-2}$)	Hydrocarbon region thickness, h (nm)	Total thickness (nm)	Bulk interface tension ($mN \cdot m^{-1}$)	Bilayer-lens contact angle (degrees)	Half bilayer tension, $\sigma/2$ ($mN \cdot m^{-1}$)	Mean channel lifetime, τ (s)	Number of channels
Control (28 mM monoolein in squalene)	7.72 ± 0.10	2.52	3.7 ± 0.1	2.29 ± 0.02	26.9 ± 0.5	2.04	30.9 ± 4.1	670
50 mM [a] *n*-decanol	7.87 ± 0.10	2.47	3.5 ± 0.4	3.29 ± 0.02	21.4 ± 0.7	3.06	18.3 ± 1.3	114
50 mM [a] *n*-hexadecanol	7.67 ± 0.10	2.54	3.7 ± 0.3	3.26 ± 0.02	25.3 ± 0.7	2.95	13.2 ± 0.3	211
18 mM [a] cholesterol	7.46 ± 0.10	2.61	3.7 ± 0.2	2.64	33.8	2.19	3.0 ± 0.8	232
0.5 mM [b] *n*-octyl-(oxyethylene)$_3$ alcohol	8.29 ± 0.10	2.35	–	2.22 ± 0.02	–	1.98 [c]	58.3 ± 16	197

[a] Oil phase concentration.
[b] Aqueous concentration.
[c] Assumes bilayer-lens contact angle = 26.9°.

tact angle through the range observed for the alcohols investigated results in a $< 12\%$ change in the value of $[(\sigma/2)^t \cos\theta^t - (\sigma/2)\cos\theta]$ plotted in Fig. 1 ('t' denotes test case).

The effects of these alcohols on bilayer thickness were small, as expected from earlier work [4]. *n*-Hexadecanol had no effect, *n*-decanol and *n*-octyl(oxyethylene)$_3$ alcohol reduced the hydrocarbon region thickness slightly and cholesterol increased h by approx. 0.1 nm. The measurements of total bilayer thickness were broadly consistent with this indicating that changes in capacity were due to thickness changes, not to an effect on the membrane dielectric constant. At the concentrations used *n*-hexadecanol and *n*-decanol increased $\sigma/2$ by approx. 1 $mN \cdot m^{-1}$, cholesterol produced a small increase and *n*-octyl(oxyethylene)$_3$ alcohol a significant reduction. The latter compound increased mean channel lifetime (τ), the others produced reduction in τ, cholesterol being by far the most effective substance.

These substances did not affect the form of the frequency distribution of channel lifetime and a satisfactory fit with one exponential time constant could always be obtained. The rate constant for channel dissociation, k_d, has therefore been calculated as $1/\tau$ for each system.

We have previously suggested that in the case of a gramicidin channel in a membrane of effective thickness greater than the hydrophobic length of the conducting dimer ($h_0 \simeq 2.2$ nm), k_d is related to the bilayer thickness and tension by

$$\ln k_d = \ln k_{d,0} + \frac{zl(\sigma/2)\cos\theta}{kT} \qquad (2)$$

$k_{d,0}$ is the rate constant in a bilayer of effective thickness equal to h_0. (The effective bilayer thickness is that with which the channel interacts and is presumed to be approximated by the hydrocarbon region thickness, h.) $(\sigma/2)\cos\theta$ is the component of the membrane tension resolved along the channel axis, which is held to be perpendicular to the plane of the bilayer (see Fig. 4 of Ref. 1). l is the perimeter of the channel along which the tension acts and z is the distance the two monomers move apart to produce the dimer to monomers transition state. k is the Boltzmann constant and T the absolute temperature. $\cos\theta$ is related to the difference between bilayer thickness and channel length $(h - h_0)$ by:

$$\cos\theta = \frac{h - h_0}{\left[(h-h_0)^2 + 4R^2\right]^{1/2}} \qquad (3)$$

where R is 0.2 nm [1].

In considering the effects of alcohols on k_d in the monoolein/squalene bilayers, the rate constant in the control system will be given by Eqn. 2. If the addition of alcohol affects only $\sigma/2$ and $\cos\theta$ then for the test case

$$\ln k_d^t = \ln k_{d,0} + \frac{zl(\sigma/2)^t \cos\theta^t}{kT} \qquad (4)$$

where the superscript 't' denotes the test case. Subtraction of Eqn. 2 from Eqn. 4 and rearranging gives

$$\ln(k_d^t/k_d) = \frac{zl}{kT}\left[(\sigma/2)^t \cos\theta^t - (\sigma/2)\cos\theta\right] \qquad (5)$$

Fig. 1 is a plot of $\ln(k_d^t/k_d)$ vs. $[(\sigma/2)^t \cos\theta^t - (\sigma/2)\cos\theta]$ using data given in Table I and set-

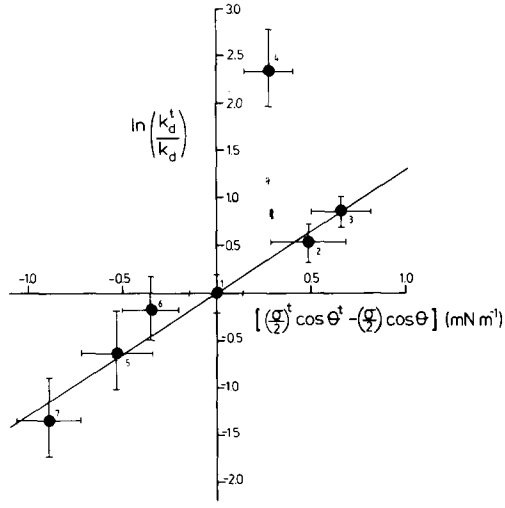

Fig. 1. A plot of $\ln(k_d^t/k_d)$ versus $[(\sigma/2)^t \cos\theta^t - (\sigma/2)\cos\theta]$. k_d is the rate constant for loss of conduction in the gramicidin channel, $\sigma/2$ is the half-bilayer surface tension and $\cos\theta$ is a function of the difference between the bilayer thickness and the conducting channel length (taken to be 2.17 nm), for a control system of monoolein/squalene (1) and various test substances (superscript 't' denotes test): *n*-decanol (2), *n*-hexadecanol (3), cholesterol (4) and *n*-octyl(oxyethylene)$_3$ alcohol (5). The plot is a test of Eqn. 5 (see text). Also shown are results from Ref. 1 for an increased monoolein concentration (6) and the addition of monopalmitolein (7). The line is a linear regression fit to the points 2, 3, 5, 6 and 7 ($r^2 = 0.96$). The error bars indicate the calculated range of values expected from the standard deviations given in Table I.

ting $h_0 = 2.17$ nm and $R = 0.2$ nm. Two other systems based on the monoolein/squalene bilayer are also included for purposes of comparison, using data from Ref. 1. These are the 133 mM monoolein in squalene and the monoolein (66 mM) plus monopalmitolein (73 mM) in squalene systems. The line on Fig. 1 was fitted to the data apart from that for cholesterol and the control system. Within experimental error the plot goes through the origin. The slope of the plot gives a value for the product zl of 5.31 nm^2. If 1 is taken as 3.77 nm [1], z is 1.41 nm, which is close to, although smaller than, the value of approx. 1.8 nm obtained in Ref. 1. The exact form of the plot in Fig. 1 is quite sensitive to the value chosen for h_0, for example 2.17 nm was found to give a better fit than 2.2 nm, which was the rounded value deduced from the previous data [1]. However, no value around 2.2 nm improves the position of the cholesterol point.

The effects of *n*-decanol, *n*-hexadecanol and *n*-octyl(oxyethylene)$_3$ alcohol on the lifetimes of gramicidin channels therefore seem explicable in terms of these compounds' effects on the surface tension and thickness of the bilayer. Both *n*-alkanols increase $\sigma/2$ but the slight thinning caused by *n*-decanol offsets some of the tension increase. The detergent *n*-octyl(oxyethlene)$_3$ alcohol reduces bilayer tension and decreases the effectiveness of that tension and hence increases channel lifetime. Cholesterol, however, produces a much larger reduction in lifetime than can be accounted for by membrane thickness and surface tension increases and must therefore influence channel lifetime in at least one other way.

It is perhaps not surprising that cholesterol should be anomalous, since the straight chain alcohols have a flexible alkyl chain, as do the lipid molecules, while most of the cholesterol molecule is rigid. As a consequence of this rigidity the packing properties of cholesterol are different from those of the lipid and *n*-alkanol chains. In particular, cholesterol does not pack into sharply curved regions of the bilayer [10] and is therefore likely to be excluded from the membrane around the conducting gramicidin dimer where $h > h_0$. If this is so the free energy of the dimer/membrane system would increase through an entropic effect. The break up of the dimer would then promote greater mixing, and the energy difference between dimer and transition state would be reduced. It can readily be calculated that at 23°C the contribution of the entropy of demixing to the free energy of the dimer/membrane system could easily be large enough to account for the observed effect of cholesterol. Channel lifetime in membranes formed from the long chain lipid monoecosenoin in squalene are also somewhat shorter than would be predicted by the thickness-tension theory (see Fig. 3B in Ref. 1). This may also arise from an increased difficulty in packing lipid molecules in a curved region of the bilayer, in this instance owing to the increased length of the chains.

The high efficacy of cholesterol is not readily attributable to an effect on membrane viscosity, since at temperatures above the lipid phase transition temperature cholesterol increases bilayer viscosity [11].

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