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THE INFLUENCE OF *n*-ALKANOLS AND CHOLESTEROL ON THE DURATION AND CONDUCTANCE OF GRAMICIDIN SINGLE CHANNELS IN MONOOLEIN BILAYERS

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The mean lifetime of gramicidin A channels in bilayers formed from monoolein and squalane was sharply reduced by the adsorption of a range of *n*-alkanols and cholesterol. Results are shown for *n*-hexanol, *n*-octanol, *n*-decanol, *n*-dodecanol, *n*-tetradecanol, *n*-hexadecanol, *n*-octadecanol and cholesterol. The longer chain *n*-alkanols were apparently more effective than the shorter members and cholesterol was the most effective of the substances examined. The single channel conductance was also affected, though to a much lesser extent than the mean channel lifetime, the *n*-alkanols producing increases and cholesterol a decrease. It is suggested that membrane fluidity changes are not likely to be primarily responsible for the reductions in channel lifetimes but that the bilayer tension, which is known to be increased by *n*-octanol, could be significant.

It is well known that the lower homologues of the straight chain aliphatic hydrocarbons and alcohols inhibit the propagation of the action potential in nerve axons [1,2]. The details of the mechanism by which this inhibition occurs are still not clear but, as far as the hydrocarbons are concerned, there are indications from recent studies that the proteins of the sodium and potassium channels are affected at least to some extent by the adsorption of the hydrocarbons into surrounding lipid. Thus, it has been shown that the thickening and increase of tension that are believed to occur when lipid bilayers adsorb hydrocarbons [2–7], together with an increase in the fluidity of the lipid chain region [8] provide reasonable qualitative explanations for changes in the Hodgkin-Huxley parameters of the sodium and potassium currents [9,10]. Whether similar types of explanation can account for the action of alcohols on nerve membranes is less obvious. One obstacle to progress has been that the effects of alcohols on well-characterized artificial membrane systems have not been as thoroughly investigated. While it has been shown that, at concentrations which block nervous impulses, aliphatic alcohols increase tension but do not greatly affect thickness in certain types of bilayer [11,26], no information is available on how the alcohols affect membrane ion permeability produced by pore-forming polypeptides such as gramicidin A. In its single channel conductance and ion selectivity the gramicidin channel bears some resemblance to the sodium and potassium channels of the nerve axon, and it was the examination of the suppression of conduction in gramicidin-containing bilayers by hydrocarbons that yielded ideas as to how these substances may be acting in the nerve [2,10–12]. With this consideration in mind, experimental results are presented

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below which show that certain normal chain alcohols and cholesterol influence the duration and conductance of gramicidin single channels.

Artificial lipid bilayers were formed from solutions of monoolein in squalane under aqueous solutions of 0.5 M KCl. The *n*-alkanols and cholesterol were added to the lipid solutions and, for systems containing *n*-octanol, *n*-decanol and *n*-dodecanol, the oil and aqueous solutions were left to equilibrate for at least 24 h prior to an experiment. Single channel lifetimes and conductances were measured essentially as described before [13]; neither parameter depended significantly on the applied potential nor on the time after membrane formation.

In Fig. 1 are shown the mean channel lifetimes for five *n*-alkanols and cholesterol as a function of their concentration in either the membrane-forming lipid solution (*n*-decanol to *n*-hexadecanol and cholesterol) or in the aqueous solution (*n*-hexanol) (inset). The dashed lines were drawn by eye through the various sets of points. It is clear that in all systems the mean channel lifetime was sharply reduced by the additive. By contrast, the single channel conductances for the same systems (Fig. 2) were not strongly affected. (The conductances for *n*-hexanol are not shown but exhibited a similar trend.) Owing to a lack of knowledge of the partition coefficients for *n*-alkanols in the present systems it is not possible to plot the results for *n*-hexanol and the higher homologues on the same concentration scale. From such partition coefficient data as is available and from some determinations of J.R. Elliott (personal communication) it appears that for *n*-decanol to *n*-hexadecanol and cholesterol, the partitioning between the lipid solution into which the alkanol is weighed originally, and the aqueous solution, is such that the nominal concentration in the former solution is not significantly affected. Similarly, for the *n*-hexanol, the nominal concentration in the aqueous phase is not greatly changed.

The *n*-hexanol concentration range covers the nerve block concentration which has been quoted as 6 mM [1]. For the other alcohols, *n*-decanol yields a nerve block concentration for the squid giant axon of approx. 0.03 mM (in the aqueous phase). The higher homologues are not obviously active on the nerve though probably because their

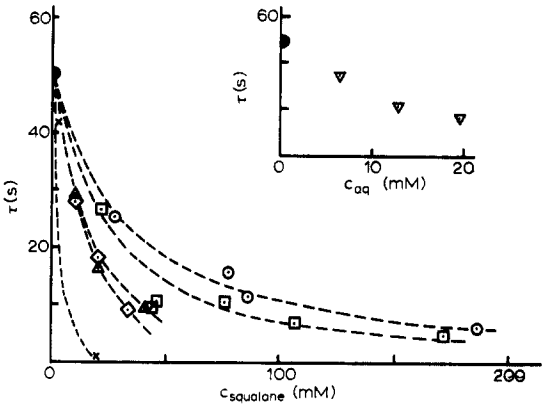

Fig. 1. The influence of *n*-alkanols and cholesterol on the mean channel lifetime (τ) for gramicidin A in monoolein-squalane bilayers. ▽, *n*-hexanol; ⊙, *n*-decanol; □, *n*-dodecanol; △, *n*-tetradecanol; ◇, *n*-hexadecanol; ×, cholesterol; ●, control. The *n*-hexanol concentration, c_{aq}, is for the aqueous phase (0.5 M KCl) while the concentrations for the other molecules are for the monoolein-squalane phase (28 mM monoolein). The channel lifetimes are the mean values for at least 200 events for each concentration, except for *n*-hexanol where only approx. 70 events were recorded. The applied potential was usually about 50 or about 100 mV. Temperature, 23±0.3°C. Each point represents the result for one experiment. Horizontal error bars should be no larger than the size of the points, as only weighings were involved. Uncertainties in τ arose mainly from the finite number of channels measured and from fluctuations in temperature. Estimates of the accuracy of the points yield approx. ±10%.

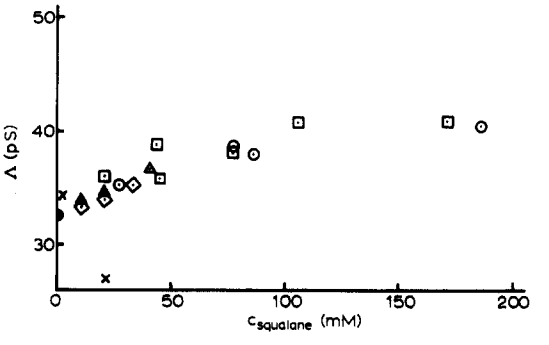

Fig. 2. The influence of *n*-alkanols and cholesterol on the single channel conductance (Λ) of gramicidin A in monoolein-squalane bilayers. ⊙, *n*-decanol; ⊡, *n*-dodecanol; △, *n*-tetradecanol; ◇, *n*-hexadecanol; ×, cholesterol; ●, control. The concentrations are for the monoolein-squalane film forming solution. The conductances are means for at least 100 events. Other experimental details are as for Fig. 1.

solubilities in water are so low. Without more precise data on the partitioning of *n*-decanol it is difficult to compare the concentrations in squalane to the aqueous nerve block concentration, but order of magnitude estimates suggest a rough correspondence.

While the experimental results show that the alkanols and cholesterol markedly affect the properties of a relatively simple ion channel, the mechanisms by which they do so are as yet not clear. It is now widely accepted that the membrane conductance produced by gramicidin A arises from a dimerization through hydrogen bonding of the monomeric species in the bilayer and that the resulting structure is a helical pore of approx. 28 Å in length, 4 Å internal and 16 Å external diameter [14,15]. The hydrocarbons (*n*-pentane to *n*-hexadecane), which adsorb primarily into the centre of the lipid chain region of the bilayer, do not appreciably affect the conductane of the channel but they considerably influence the equilibrium constant for dimerization of the polypeptide. Thus, as the chain length of the hydrocarbon decreases, and its adsorption increases, the association rate constant goes down by orders of magnitude, and the dissociation rate constant increases, though to a much lesser extent [12,13,17,18]. The change in the equilibrium constant was accounted for in terms of the surface free energy of deformation of the membrane needed for channel formation when the bilayer thickness exceeded the channel length [12]. No attempt was made to account for the changes in the individual rate constants.

The alkanols and cholesterol, being polar molecules, must compete much more strongly than the hydrocarbons for space in the surface of the bilayer. Estimates of the standard free energy of adsorption of alkanols from hydrocarbons to hydrocarbon/water interfaces [19] suggest that the alkanol is more strongly adsorbed than the hydrocarbon by a factor of approx. 500. The increase in the gramicidin channel conductance could therefore be a surface effect associated with the replacement of acylglycerol by hydroxyl groups near the channel entrances. This explanation obviously does not account for the decrease in conductance produced by cholesterol, but the very different structure of the hydrophobic part of this molecule may be a contributory factor.

In contrast to the hydrocarbon experiments referred to above, no very accurate estimate of the changes in the equilibrium constant for dimer formation seemed feasible for the alkanols. Experiments in which *n*-octanol was added to monoolein-squalane bilayers containing pyromellityl gramicidin showed the membrane conductance changes to be well within an order of magnitude for nerve-block concentrations ($\leq$ 1.0 mM in the aqueous phase) (Elliott, J.R., personal communication). This result may be compared with the change of four orders of magnitude found for nerve-blocking concentrations of *n*-pentane or *n*-hexane [12]. It appears therefore that, relatively, the alkanols do not greatly influence the equilibrium constant for gramicidin dimerization, although this conclusion is subject to the assumption that the adsorption of the alkanol into the bilayer does not significantly affect the adsorption of the pyromellityl gramicidin.

In reducing the mean channel lifetime (or increasing the dissociation rate constant) the alkanols and cholesterol resemble the lower hydrocarbons [12,20–22]. The results in Fig. 1 suggest that the efficacy of the alkanols increases with chain length and that cholesterol is especially effective. Without a knowledge of the activity coefficients for the systems in question (these have not been reported and would be quite difficult to measure) the picture will remain unclear quantitatively, but it is very unlikely that the activity coefficients for the different alkanols and cholesterol vary from one to the other sufficiently to change the apparent order of efficacy. A further argument that the chain length effect is genuine, and which to some extent circumvents the lack of knowledge of the activity coefficients, stems from Fig. 3 where log (mean channel lifetime) is plotted against channel conductance. Thus if, as suggested, it is primarily the surface density of hydroxyl groups which affects the single channel conductance, then a given conductance should correspond to a particular membrane concentration of alkanol, independently of the alkanol chain length. It follows that if all chain lengths had been equally effective, all the points in Fig. 3 would have fallen on the same line, rather than showing essentially the same variation as in Fig. 1.

Cholesterol and *n*-alkanols of chain length

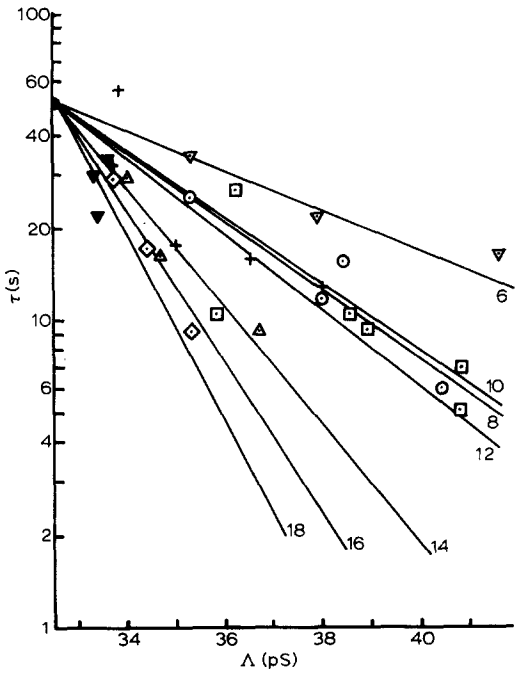

Fig. 3. Log_{10} [mean channel lifetime (τ)] versus single channel conductance (Λ) for gramicidin A in monoolein-squalane bilayers in the presence of different concentrations of *n*-alkanols. ▽, *n*-hexanol; +, *n*-octanol; ⊙, *n*-decanol; ⊡, *n*-dodecanol; △, *n*-tetradecanol; ◇, *n*-hexadecanol; ▼, *n*-octadecanol. The linear regression lines through the points for the various systems are labelled with the number of carbon atoms in the *n*-alkanol. The data are those of Figs. 1 and 2 together with data for *n*-octanol and *n*-octadecanol which could not conveniently be displayed on these figures.

greater than ten are thought to restrict the segmental motion of the lipid chains in bilayers [23,24]. It thus seems unlikely that the increases observed in the dissociation rate constant for gramicidin are caused by 'fluidity' changes. For the hydrocarbon systems mentioned above, both Neher and Eibl [21] and Rudnev et al. [22] have produced evidence that the membrane tension is a significant factor in determining the dissociation rate constant. That this should be so seems reasonable if only because the equilibrium constant has been shown to depend on the tension [12]. It is also reasonable physically because if the channel length is smaller than the membrane thickness (such that the membrane is deformed or dimpled in the vicinity of the channel) the membrane tension will contribute to the stresses which tend to disrupt the channel. It has been shown that *n*-octanol increases the tension of a monoolein-squalane bilayer [11] and other *n*-alkanols and cholesterol should also do so. (If log τ is a roughly linear function of membrane surface tension [21,22] it is possible that the linearity of the plots of Fig. 3 arises from the channel conductance (Λ) and the membrane concentration of alkanol being not only related linearly to each other but also to the membrane surface tension.) Provided that there is an appropriate mis-match between channel length and membrane thickness, the proposed tension mechanism could also account for the present observations. As already mentioned, the length of the gramicidin dimer is approx. 28 Å. The thickness of the hydrocarbon chain region of the monoolein-squalane bilayers is, from capacity measurements, approx. 25 Å [11], whereas the total thickness, from optical reflectance, is approx. 37 Å [25]. The significance of these dimensions is not easy to assess since the effective hydrophobicity of the external surface of the gramicidin dimer is important and could vary with position along the length of the channel. It is clear, however, that the required thickness mismatch could exist. At the same time it cannot be ruled out that the alcohols affect the strength of the hydrogen bonding between the two parts of the channel dimer.

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