Lysophospholipids Modulate Channel Function by Altering the Mechanical Properties of Lipid Bilayers

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ABSTRACT Lipid metabolites, free fatty acids and lysophospholipids, modify the function of membrane proteins including ion channels. Such alterations can occur through signal transduction pathways, but may also result from "direct" effects of the metabolite on the protein. To investigate possible mechanisms for such direct effects, we examined the alterations of gramicidin channel function by lysophospholipids (LPLs): lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylserine (LPS), and lysophosphatidylinositol (LPI). The experiments were done on planar bilayers formed by diphytanoylphosphatidylcholine in *n*-decane a system where receptor-mediated effects can be excluded. At aqueous concentrations below the critical micelle concentration (CMC), LPLs can increase the dimerization constant for membrane-bound gramicidin up to 500-fold (at 2 μM). The relative potency increases as a function of the size of the polar head group, but does not seem to vary as a function of head group charge. The increased dimerization constant results primarily from an increase in the rate constant for channel formation, which can increase more than 100-fold (in the presence of LPC and LPI), whereas the channel dissociation rate constant decreases only about fivefold. The LPL effect cannot be ascribed to an increased membrane fluidity, which would give rise to an increased channel dissociation rate constant. The ability of LPC to decrease the channel dissociation rate constant varies as a function of channel length (which is always less than the membrane's equilibrium thickness): as the channel length is decreased, the potency of LPC is increased. LPC has no effect on membrane thickness or the surface tension of monolayers at the air/electrolyte interface. The bilayer-forming glycerolmonooleate does not decrease the channel dissociation rate constant. These results show that LPLs alter gramicidin channel function by altering the membrane deformation energy, and that the changes in deformation energy can be related to the molecular "shape" of the membrane-modifying compounds. Similar alterations in the mechanical properties of biological membranes may form a general mechanism by which one can alter membrane protein function.

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INTRODUCTION

Lysophospholipids (LPLs) can modulate the function of membrane proteins, e.g., inward rectifier potassium channels (Kiyosue and Arita, 1986); voltage-dependent sodium channels (Burnashev, Undrovinas, Fleidervish, and Rosenshtraukh, 1989); $K_{(ATP)}$ channels (Eddlestone and Ciani, 1991); and Na^+,K^+-ATPase (Oishi, Zheng, and Kuo, 1990). More generally, widely diverse types of membrane proteins can be modified "directly" by a variety of amphipathic lipid metabolites with seemingly little specificity: diacylglycerols (Hockberger, Toselli, Swandulla, and Lux, 1989); free fatty acids (Ordway, Walsh, and Singer, 1989; Hwang, Guggino, and Guggino, 1990; Wallert, Ackerman, Kim, and Clapham, 1991; Shimada and Somlyo, 1992); acylcarnitines (Adams, Cohen, Gupte, Johnson, Wallick, Wang, and Schwartz, 1979; Sato, Kiyosue, and Makoto, 1992); and eicosanoids (Buttner, Siegelbaum, and Volterra, 1989). Receptor-mediated effects do not appear to be involved, and the mechanisms underlying the functional alterations are not understood. The changes in function occur at fairly high (micromolar) aqueous metabolite concentrations, however, which may be sufficient to affect the physical properties of the bilayer in which the proteins are imbedded. Platelet-activating-factor (PAF), for example, at nanomolar concentrations exerts its effects through receptor-mediated mechanisms (Honda et al., 1991). But at pathophysiological (μM) concentrations, PAF can modify the function of gramicidin channels in lipid bilayer membranes (Sawyer, Koeppe, and Andersen, 1989), an effect that cannot be due to receptor-mediated mechanisms. These observations raise the question, to what extent "direct" effects of amphipathic lipid metabolites (and other amphipathic compounds) on membrane protein function should be ascribed to binding to the membrane proteins per se and to what extent these compounds could act more indirectly by altering the physical (mechanical) properties of the host bilayer?

A change in the mechanical properties of a bilayer could modify the function of incorporated proteins by changing the free energy difference between different conformational states of the protein (Gruner, 1991; Andersen, Sawyer, and Koeppe, 1992; Keller, Bezrukov, Gruner, Tate, Vodyanoy, and Parsegian, 1993). This is because the energetic cost of exposing hydrophobic groups to water will tend to ensure that the hydrophobic thickness of the lipid bilayer immediately adjacent to the membrane-spanning protein will match the hydrophobic length of the proteins hydrophobic exterior (Hendry, Urban, and Haydon, 1978; Elliott, Needham, Dilger, and Haydon, 1983; Mouritsen and Bloom, 1984). A protein conformational change that involves a change in (the length of) the proteins hydrophobic exterior surface will lead to a deformation of the adjacent bilayer. As the equilibrium distribution between conformational states of a protein is determined by the total free energy difference between these states, the energetic cost of the membrane deformation will contribute toward determining the equilibrium distribution between conformational states of the protein.

The mechanical properties of a bilayer, will among other factors, be related to the "shape" of the molecules in the membrane. Phospholipids, which constitute the majority of the lipids in a cell membrane, have an approximately cylindrical molecular shape: the cross-sectional area of their polar head group is similar to that

of the acyl chains in the core of the membrane. LPLs are cone shaped, as their polar head groups have a larger cross-sectional area than that of the single acyl chain, and promote the formation of curved (or nonbilayer) structures (Carnie, Israelachvili, and Pailthorpe, 1979; Cullis and de Kruijff, 1979). If these compounds accumulate in a membrane they will tend to alter the spontaneous curvature of the membrane (monolayer) and, more generally, the energetic cost of a membrane deformation—and thus the equilibrium distribution among different conformational states of membrane proteins (Gruner, 1989, 1991; Andersen et al., 1992; Gibson and Brown, 1993; Keller et al., 1993).

To investigate the interactions between lipid metabolites and membrane proteins, we examined the effect of LPLs on gramicidin channels incorporated into planar bilayers. The linear gramicidins form transmembrane channels that are formyl-NH-terminal-to-formyl-NH-terminal dimers of right-handed $\beta^{6.3}$-helices (for recent reviews on gramicidin channels see Andersen and Koeppe [1992], Killian [1992] and Busath [1993]. The channel structure is known at near-atomic resolution, which makes it the best understood ion channel. In this system, receptor-mediated mechanisms can be excluded. Further, the structural features of gramicidin channels makes them particularly useful to examine the mechanical aspects of membrane-protein interaction because gramicidin form ion channels by the transmembrane association of one monomer from each monolayer (O'Connell, Koeppe, and Andersen, 1990).

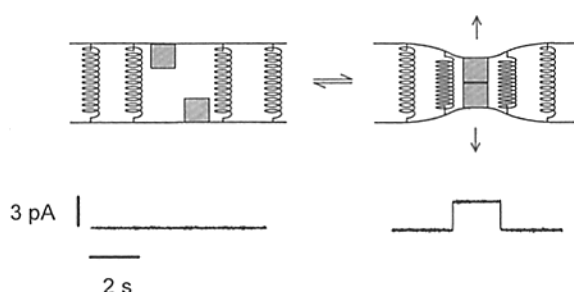

FIGURE 1. Schematic representation of gramicidin channel formation by the transmembrane association of two monomers. The upper part of the figure shows monomers (*left*) and a membrane-spanning dimer (*right*). The lower part of the figure shows the corresponding current signals. Channel formation is associated with a membrane deformation. The energetic cost of the membrane deformation will contribute to the overall channel stability. The elastic properties of the bilayer are visualized as localized springs (Mouritsen and Bloom, 1984).

Gramicidin channel formation is associated with a membrane deformation (Fig. 1), in which the bilayer surrounding the channel is compressed and the monolayers bend towards each other, to adjust the membrane's hydrophobic thickness to the length of channel's hydrophobic exterior surface (Elliott et al., 1983; Huang, 1986; Helfrich and Jakobsson, 1990). Alterations in a bilayer's ability to adjust to the channel will alter the equilibrium constant for gramicidin channel formation.

We examined the effect of four LPLs: lysophosphatidylcholine (LPC); lysophosphatidylinositol (LPI); lysophosphatidylethanolamine (LPE) and lysophosphatidylserine (LPS). These compounds were chosen because they allow for an evaluation of the importance of the molecular shape (LPC and LPI have larger head groups than LPE and LPS) and charge (LPI and LPS are negatively charged, whereas LPC and LPE are uncharged). Moreover, LPC and LPI alter the function of the $K_{(ATP)}$ channel, whereas LPE and LPS have no effect (Eddlestone and Ciani, 1991).

All four LPLs modify the function of gramicidin channels in planar bilayers. Their effects are qualitatively similar: the channel appearance rate and average duration increase, and the standard free energy for channel formation is decreased. The relative potency of the LPLs is correlated with the size of their polar head groups (their molecular shape). The magnitude of the increase in channel duration varies as a function of the channel length. These results strongly suggest that the LPLs act by decreasing the energetic cost of the membrane deformation. This may be a general mechanism whereby amphipathic lipid metabolites (and other nonpolar/amphipathic molecules) can modify membrane protein function.

Some of this material has appeared in preliminary form (Lundbæk and Andersen, 1993).

MATERIALS AND METHODS

The gramicidin analogues were a gift from Dr. Roger E. Koeppe II (Department of Chemistry and Biochemistry, University of Arkansas). Gramicidin A (gA) was purified from the naturally occurring mixture of gramicidin A, B, and C by HPLC (Koeppe and Weiss, 1981). *Endo*-Gly^{0a}-gramicidin C (*endo*-Gly^{0a}-gC) and des-Val^{1}-gramicidin C (des-Val^{1}-gC) were synthesized as described previously (Durkin, Providence, Koeppe, and Andersen, 1993). The naturally occurring gramicidins have the sequence (Sarges and Witkop, 1965; Gross and Witkop, 1965): formyl-L-Xxx^{1}-D-Gly^{2}-L-Ala^{3}-D-Leu^{4}-L-Ala^{5}-D-Val^{6}-L-Val^{7}-D-Val^{8}-L-Trp^{9}-D-Leu^{10}-L-Yyy^{11}-D-Leu^{12}-L-Trp^{13}-D-Leu^{14}-L-Trp-^{15}-ethanolamine, where Xxx is either Val or Ile and Yyy is Trp, Phe or Tyr in gramicidin A, B, or C, respectively. gA has the sequence formyl-Val^{1}–Trp^{11}–ethanolamine. The two synthetic gramicidin analogues have the sequences: formyl-Gly^{0a}-L-Val^{1}-Tyr^{11}-ethanolamine, (*endo*-Gly^{0a}-gC) and formyl-Gly^{2}–Tyr^{11}–ethanolamine, (*des*-Val^{1}-gC).

Diphytanoylphosphatidylcholine (DPhPC) from Avanti Polar Lipids, Inc. (Alabaster, AL) was further purified by ion-exchange chromatography (Andersen, 1983). *n*-decane was from Wiley Organics (Columbus, OH) and was used as supplied. The membrane-forming solution was usually DPhPC in *n*-decane (2–3% wt/vol). In a few experiments, 1-monooleoyl-rac-glycerol (GMO) was added to the DPhPC/*n*-decane membrane-forming solution at a DPhPC/GMO molar ratio of 1/1.

The LPLs were from Avanti Polar Lipids, Inc.; GMO was from Sigma Chemical Company, (St. Louis, MO). The chemical composition and origin of these compounds are listed in Table I.

NaCl, analytical grade from EM Science (Cherry Hill, NJ), was roasted at 500°C for 24 h and stored over $CaSO_4$ in an evacuated dessicator. Water was deionized Milli-Q water (Millipore Corp., Bedford, MA). Ethanol was from U.S. Industrial Chemicals (Tuscola, IL).

Experimental Procedures

Planar bilayers (large membranes) were formed across a ~1.6-mm diam hole in a Teflon partitioning separating two Teflon chambers. Each chamber contained 5 ml unbuffered 1.0 M NaCl at 25 ± 1°C. In initial experiments it was noted that the quantitative effect of LPLs

decreased as the amount of membrane-forming solution used was increased. In the experiments reported here, care was taken to minimize the total amount of membrane-forming solution used; a maximum of 10 μl of the bilayer-forming solution (usually ~2–3 μl) was used in each experiment.

Stock solutions of gramicidin (0.15–15 nM) and LPLs (2.5–5 mM) were made up in ethanol, and small aliquots were added to both electrolyte solutions during vigorous stirring. The LPL solutions were made up every 2 mo. A maximum of 80 μl ethanol (as solvent) was added to each chamber. (This amount of ethanol has no effect on channel function, O. S. Andersen, unpublished observations.) The aqueous concentrations of LPLs and gramicidin will be given as the nominal concentrations, based upon the total amount of compound added.

Single-Channel Experiments

The bilayer-punch method (Andersen, 1983) was used to isolate a small area (~30 μm pipette diam) of the large membrane and the single-channel current activity was recorded at 200 mV. The current signal was amplified using an Axopatch-1B (Axon Instruments, Foster City, CA). The current signal was filtered (30–150 Hz) with an 8 pole Bessel filter (Frequency Devices, Haverhill, MA) before input to an A/D converter in a PC/AT compatible 80486 computer.

TABLE I

Fatty Acid Composition and Origin of the Membrane Modifiers Used in this Study

LPL	Origin	Fatty acid composition		
		16:0	18:0	18:1
			%	
LPC	chicken egg	60	30	5
LPI	bovine liver	5	80	15
LPE	chicken egg	40	55	
LPS	bovine brain	5	80	5
GMO	synthetic			99

The data for the LPLs are based on information from Avanti Polar Lipids, Inc.

Transitions were detected on-line as described by Andersen (1983). Single-channel durations (τ) and conductances (g) were determined as described by Sawyer et al. (1989); Durkin, Koeppe, and Andersen (1990). In control experiments (without LPL), the nominal gramicidin concentration was 2–10 pM. The addition of LPLs leads to a large increase in channel activity, and measurements in the presence of LPLs were done in separate experiments at lower gramicidin concentrations (~1 pM). In these experiments, the electrolyte solutions were stirred for 2–5 min after addition of the LPLs, and the measurements were begun.

Channel Activity (Membrane Conductance)

The effect of LPLs on channel activity (number of conducting channels in a membrane) was determined in a separate set of experiments done on the large membranes (~1.6 mm diam). Potentials of 2–20 mV were applied across a large membrane and the resulting current (filtered at 0.1 Hz) measured (see Results).

Specific Capacitance

The capacitance of the bilayers was measured by applying a sawtooth potential across a large membrane. The bilayer area was determined using a microscope with a calibrated reticule. LPC was then added to the electrolyte solution and the measurement repeated after stirring the chamber for 30 min.

Surface Tension

The surface tension of DPhPC monolayers at the air/electrolyte interface was measured by the Du Noüy ring method (Harkins and Jordan, 1930). (We thank Drs. S. A. Simon and E. M. Arnett of Duke University for assistance with the measurements.) A horizontal platinum ring (thickness 0.35 mm, circumference 6 cm) was submerged in 10 ml 1.0 M NaCl placed in a beaker with a diameter of 4.5 cm (25 ± 1°C). 4 μl DPhPC dissolved in chloroform (29.5 mM) was added to the surface of the solution. After 10 min, when the chloroform had evaporated, the ring was raised through the surface. The surface tension of the monolayer was determined from the force required to raise the ring. The effects of LPLs were determined in separate experiments where either 4 μM LPC or LPE were added to the electrolyte subphase before spreading the monolayer. The surface tension was determined as in the control experiments.

RESULTS

Single-Channel Experiments: Gramicidin A

Fig. 2 shows the effect of LPC on the behavior of gramicidin A (gA) channels in a DPhPC bilayer. When LPC is added to the electrolyte solution bathing the bilayer,

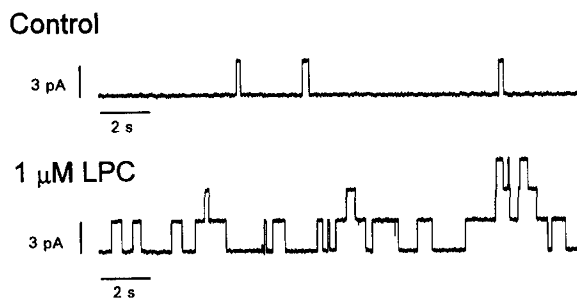

FIGURE 2. The effect of LPC on gA channels in a DPhPC/*n*-decane bilayer. Single-channel current traces from the same large membrane before (*top*) and after (*bottom*) the addition of 1 μM LPC to the aqueous solution. The calibration bars denote 5 pA (*vertically*) and 2.5 s (*horizontally*). 200 mV, 200 Hz, 1 M NaCl, 25°C.

there is a pronounced increase in channel activity: both the channel appearance rate and the average duration (τ) of the channels are increased. In addition, the single-channel conductance (g) is decreased.

The effects of LPC on τ and g are quantified in Fig. 3. τ increased from 550 ms in the absence of LPC to 2,790 ms at 4 μM LPC. In both the absence and the presence of LPC, the single-channel duration histograms can be described by single-exponential distributions (Fig. 3 *A*). Similarly, g decreases in a [LPC] dependent manner: from 15.0 pS in the control experiments to 11.4 pS at 4 μM LPC. The changes in g were quantified using current transition amplitude histograms (Fig. 3 *B*).

g and τ do not vary systematically with time during the experiments (that may last >3 h). This is shown in Fig. 4, which shows the relative variations in g and τ in the absence and presence of LPC. The time invariance of τ indicates that the mole fractions of decane and LPC in the membrane are constant for the duration of the experiments.

Qualitatively, all four LPLs produce similar changes in τ, but their quantitative effects differ (Fig. 5). For all four LPLs, τ is increased in a concentration-dependent manner, and the effect tends to level off at the higher concentrations. LPC and LPI

are the most potent: at 4 μM they increase τ five- and fourfold, respectively. LPE and LPS at the same concentration produce only a threefold increase in τ.

The changes in g are shown in Fig. 6. Again, all four LPLs have similar effects: they produce a concentration-dependent decrease in g. At the lower LPL concentrations, g decreases as an approximately linear function of [LPL]. As for the changes in channel duration, the conductance changes tend to level off at the higher [LPL]. At 4 μM the zwitterionic (but net neutral) LPC and LPE decrease g from 15.0 to 11.4 and 9.3 pS,

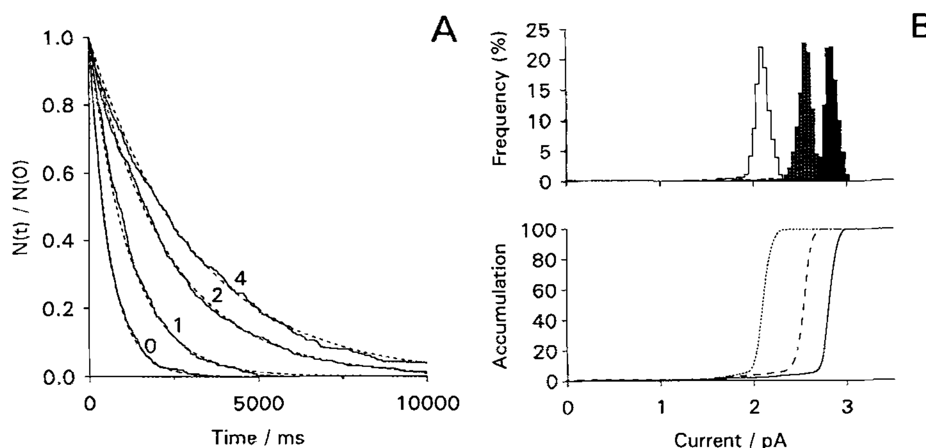

FIGURE 3. (*A*) Normalized survivor histograms for gramicidin A channels in DPhPC/*n*-decane membranes. The solid curve labeled *0* denotes results obtained in the absence of LPC; the solid curves labeled *1*, *2*, and *4* denote results obtained after the addition of 1, 2, and 4 μM LPC to the aqueous solution. The interrupted curves denotes the best fit of a single exponential distribution to the results: $N(t)/N(0) = \exp(-t/\tau)$, where $N(0)$ and $N(t)$ denote the number of channels at time zero and t. In the control experiment (0), τ was 600 ms, $N(0) = 725$. With 1, 2, 4 μM LPC, τ was 1170, 2300, 3080 ms, respectively, and $N(0)$ was 475, 552, 419. (*B*) Current transition amplitude histograms (*top*) and distribution functions (*bottom*) for gA channels in DPhPC/*n*-decane membranes. The filled histogram and solid distribution function are based upon 1,490 events from five measurements in a control experiment, the main distribution contains 95% of events. The average current was 2.84 ± 0.07 pA (mean ± SD). The cross-hatched histogram and interrupted curve are based upon six measurements in an experiment with 1 μM LPC (1320 events, 94% in main peak). The average current was 2.57 ± 0.07 pA (mean ± SD). The "empty" histogram and stippled distribution function are based upon five measurements in an experiment with 4 μM LPC. The average current was 2.12 ± 0.07 pA (1,682 events, 96% in main peak). 200 mV, 1 M NaCl, 25°C.

respectively. The negatively charged LPI and LPS have less effect, they decrease g only to 12.7 and 13.4 pS. The decrease in g may be due to an increase in the interfacial dipole potential around the channel (see Discussion). That the effect of the anionic LPI and LPS is less than that of LPC and LPE is presumably due to the fact that the former will import a net negative charge to the membrane (which will tend to increase the conductance of the cation-selective channel [Apell, Bamberg, and Läuger, 1979]).

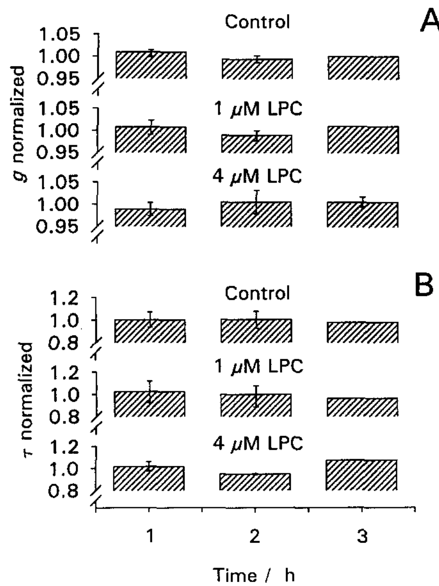

FIGURE 4. The time course of the average single-channel conductance (A) and duration (B) in control experiments and in experiments with 1 and 4 μM LPC. To counter the effect of day-to-day variations the results are normalized to the average value (over 3 h) for each experiment. The results show the geometric average ± range of these normalized values in 0, 1, and 4 μM LPC. (The results for 3 h are in most cases based on single experiments.) In the control experiments, zero time was when gA was added. In experiments with LPC, the zero time was when LPC was added. DPhPC/n-decane, 200 mV, 1 M NaCl, 25°C.

Gramicidin Channel Activity

The effect of LPL on channel activity (the average number of conducting channels in a membrane) was determined by measuring the change in the conductance (G) of a "large" membrane (area ~2 mm^2) when LPLs were added to the aqueous solution. Gramicidin (10–30 pM) was added during continuous stirring to both electrolyte solutions bathing a DPhPC/n-decane membrane. The formation of conducting channels was observed as an increase in G to a new stable level in ~40 min (Fig. 7 A). When a stable conductance was reached, the stirrer was turned off to allow more

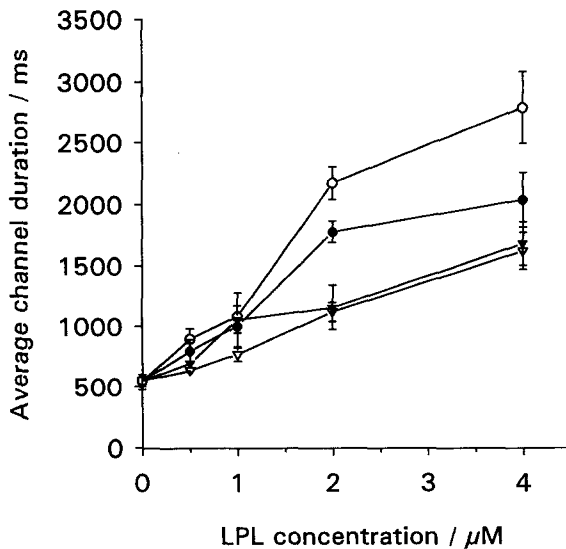

FIGURE 5. The effect of the different LPLs on the average duration of gA channels. Each point represents the mean ± range of at least two independent experiments. (□) Control; (○) LPC; (●) LPI; (▽) LPE; (▼) LPS. DPhPC/n-decane, 200 mV, 1 M NaCl, 25°C.

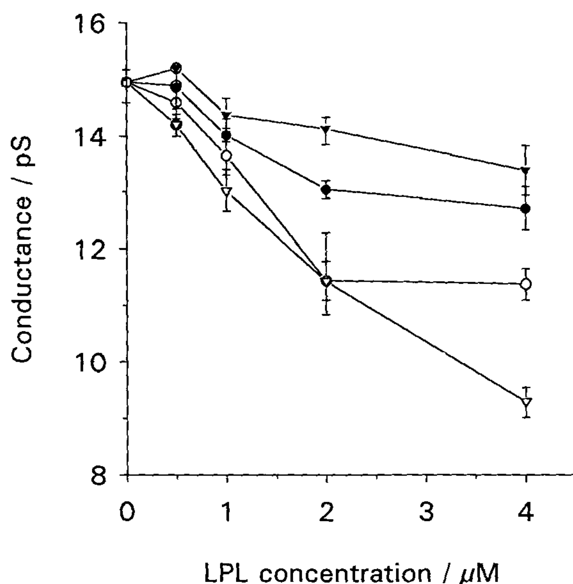

FIGURE 6. The effect of LPLs on the conductance of gA channels. Each point represents the mean ± range of at least two independent experiments. (□) Control; (○) LPC; (●) LPI; (▽) LPE; (▼) LPS. DPhPC/*n*-decane, 200 mV, 1 M NaCl, 25°C.

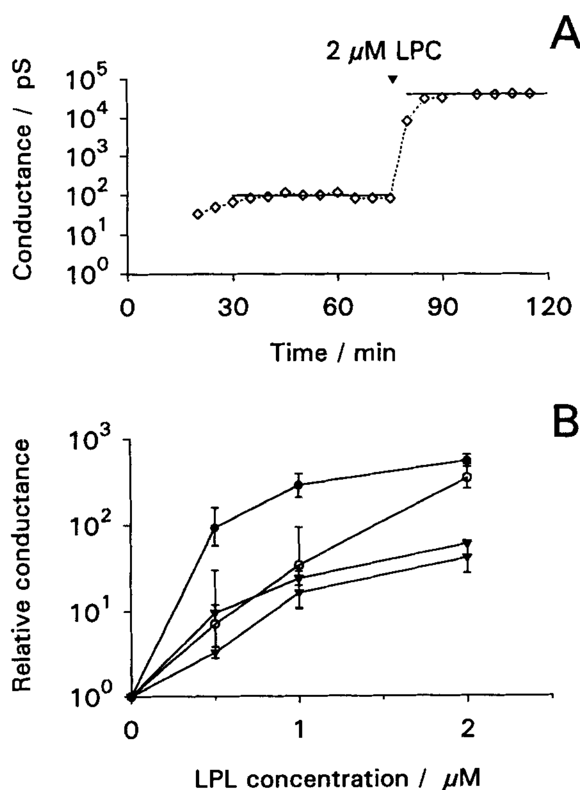

FIGURE 7. The effect of LPC on the conductance of a DPhPC/*n*-decane membrane in the presence of gA. (*A*) Results from a single experiment in which gA, is added at time zero (during continuous stirring) to the electrolyte solutions bathing a DPhPC/*n*-decane membrane. After ~45 min, a steady state conductance of gA is reached. After 76 min, 2 μM LPC is added to the electrolyte solutions and the membrane conductance rises to a new steady level in ~15 min. 20 mV, 1 M NaCl, 25°C. (*B*) Effect of different LPLs on the conductance of DPhPC/*n*-decane membranes in the presence of gA. Each point in the figure represents the geometric mean ± range of (G_L/G_C) in at least two independent experiments. (○) LPC; (●) LPI; (▽) LPE; (▼) LPS. DPhPC/*n*-decane, 2–20 mV, 1 M NaCl, 25°C.

accurate measurements. In experiments where no LPL was added, G did not change systematically for up to 90 min after the stirring had been discontinued. (This relatively constant conductance differs from previous reports [e.g., Kemp and Wenner, 1976]; it is probably due to the very low gramicidin concentrations used in the present experiments.) The chosen LPL (0.5–2 μM) was then added to both electrolyte solutions during continuous stirring, and G increased to a new stable level in ~30 min. The stirrer was turned off again and the current would usually remain stable over the next 40 min. (Results with 4 μM LPL could not be obtained, as the membrane stability was reduced at concentrations above 2 μM.)

Fig. 7 *B* shows the effect of the LPLs on G. 2 μM LPC and LPI increase G some 300- and 500-fold, respectively, whereas LPE and LPS at that concentration increase G only 60- and 40-fold. The membrane conductance increases shows a similar pattern as the increases in channel duration: LPC and LPI are much more potent than LPE and LPS.

The increase in G is due to an increase in the number of conducting channels. The number of conducting channels (N) in a membrane can be related to G by:

$$N = (G - G_b)/g, \tag{1a}$$

where G_b denotes the background conductance of the unmodified membrane (in the absence of gramicidin). $G \gg G_b$, and one can simplify

$$N \approx G/g. \tag{1b}$$

The ratio of the number of channels in the control situation, N_C, and in the presence of LPLs, N_L, is given by:

$$N_L/N_C \approx (G_L/G_C)\cdot(g_C/g_L) \tag{2}$$

where we use the subscript C to signify a control value and the subscript L to signify a value obtained in the presence of LPL. Based on the results in Figs. 6 and 7 *B*, 2 μM LPC and LPI increase the number of conducting channels ~500-fold (450- and 630-fold, respectively) whereas, LPE and LPS increase this ratio by only ~60-fold (80- and 40-fold, respectively).

The conductance of lipid bilayers (the background conductance in absence of gramicidin) is increased by LPLs (van Zutphen and van Deenen, 1967). But this is not a major contribution to the conductance increases seen when LPLs are added to gramicidin-containing membranes. Before LPL addition, the conductance in the presence of gramicidin was ~250 pS (range 100–500 pS), which is 10-fold larger than the background conductance of the membranes (~20 pS, range 10–50 pS). Addition of 2 μM LPC to gramicidin-containing membranes increased the conductance ~300-fold, which should be compared to an only ~50-fold increase for unmodified DPhPC/*n*-decane membranes. Even if the background conductance increased 100-fold after addition of 2 μM LPC, the change in background conductance would contribute less than 5% to the total conductance increase.

The increased channel activity could be due to an increased channel forming ability of gramicidin already in the lipid bilayer, to an LPL-mediated increase in the adsorption of gA to the bilayer, or to combination of these effects. To determine to what extent the conductance increase might result from an increased adsorption of

gramicidin, experiments were done in which gramicidin was added directly to the membrane-forming DPhPC/n-decane solution (gramicidin/DPhPC molar ratio $1/10^7$, cf. Sawyer et al. [1989]). If the effects of the LPLs on the membrane conductance primarily were due to an increased adsorption of gramicidin molecules to the membrane, one would expect the conductance increase to be smaller and occur over a shorter time in experiments where the gramicidin is added directly to the membrane-forming solution.

In these experiments, the average conductance of the gramicidin-doped membrane was 400 pS (range 200–600 pS), and addition of 1.0 μM LPC to both electrolyte solutions increased the conductance ~20-fold in ~30 min. In the corresponding standard experiments, the membrane conductance rose ~30-fold in ~25 min. The conductance increase therefore is due primarily to an increased channel-forming ability and not to an increased adsorption of gramicidin to the membrane.

Effect of GMO on Gramicidin Channels in DPhPC Membranes

Like the LPLs, GMO has a single acyl chain; but GMO is a bilayer-forming monoglyceride because its polar head group occupies approximately the same cross-sectional area as the single acyl chain. To test whether the effect of the LPLs is related to their (noncylindrical) cone shape, we examined the effect of GMO on gA channels. Addition of GMO to a DPhPC bilayer should not decrease the cost of bilayer deformation and therefore, not increase τ. We measured τ in experiments where GMO had been added to the membrane-forming solution at a GMO/DPhPC molar ratio of 1:1. (When GMO is added through the electrolyte solution it does not incorporate into the bilayer; L. L. Providence, J. A. Lundbæk, and O. S. Andersen, unpublished observations.) In the presence of GMO, τ was ~410 ms (range 390–430 ms), which is slightly less than in DPhPC/n-decane membranes (and comparable to the value in GMO/n-decane bilayers [Sawyer et al., 1989]). That the bilayers were mixed DPhPC/GMO membranes was verified by monitoring g, which was 20 pS. (g = 15 pS in DPhPC/n-decane and 25 pS in GMO/n-decane membranes.) For comparison, when LPC was added to the membrane-forming solution at a LPC/DPhPC molar ratio of 1:4, τ was 1400 ms.

Membrane Thickness

The average duration of gramicidin channels varies as a function of the bilayer thickness (Hladky and Haydon, 1972, 1984; Kolb and Bamberg, 1977; Rudnev, Ermishkin, Fonina, and Rovin, 1981), and LPLs could exert their effect on channel function by altering the bilayer thickness. To examine whether this could be a major cause of the activity increase, we measured the effect of LPLs on the specific capacitance of DPhPC/n-decane bilayers. The specific capacitance of an unmodified DPhPC/n-decane membrane is 0.40 ± 0.04 μF/cm^2 (mean ± SD, n = 5), corresponding to an average thickness of the hydrophobic core of ~4.7 nm (assuming a dielectric constant of 2.1). After addition of 2 μM LPC to the electrolyte solution, the specific capacitance was 0.41 μF/cm^2 ± 0.02 (mean ± SD, n = 5), which is not significantly different from the value measured in the absence of LPLs. The effects of the LPLs cannot be ascribed to a change in the membrane thickness.

Surface Tension

LPLs could alter channel duration and channel appearance rates by altering the surface tension of the membranes (cf. Hladky and Haydon, 1984). To investigate whether this could be a major factor, we determined the effect of LPC and LPE on the surface tension of DPhPC monolayers, using the Du Noüy ring method (Harkins and Jordan, 1930). The surface tension of DPhPC monolayers spread on 1 M NaCl was 25.9 ± 1.6 mN/m (mean ± SD, $n = 4$). This value is comparable to the surface tension of dimyristoylphosphatidylcholine monolayers at the water/air interface at 25°C (MacDonald and Simon, 1987). In the presence of 4 μM LPC or LPE the surface tension was 26.2 ± 0.4 mN/m (mean ± SD, $n = 3$) and 25.1 ± 0.4 mN/m (mean ± SD, $n = 3$), respectively. At these concentrations, the LPLs do not induce changes in the surface tension of DPhPC monolayers that are sufficient to account for the observed changes in channel appearance rate and duration (see Discussion).

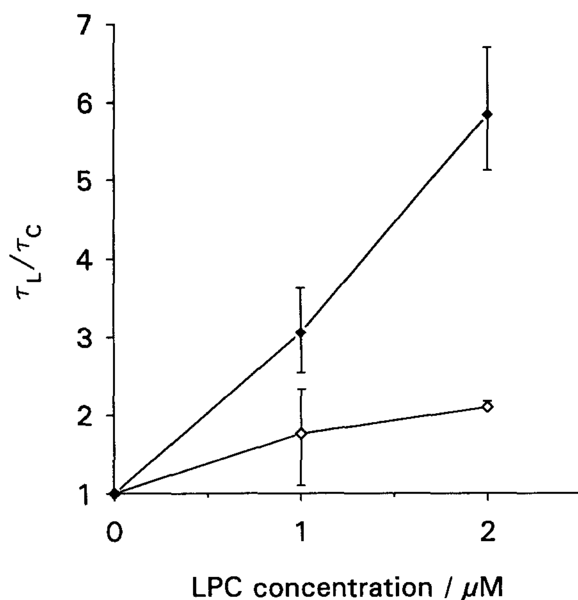

FIGURE 8. The effect of LPC on the average duration of gramicidin channels that have different lengths. The average durations of *endo*-Gly^{0a}-gC and *des*-Val^{1}gC are shown (relative to the individual control values in the absence of LPC). Each point represents the mean ± range of at least two experiments. The duration of the longer *endo*-Gly^{0a}-gC channels increased from 410 ms (range 405–415 ms) in control experiments to 862 ms (range 831–892 ms) in the presence of 2 μM LPC. The duration of the shorter *des*-Val^{1}-gC channels increased from 46 ms (range 38–53 ms) in control experiments to 267 ms (range 234–306) in the presence of 2 μM LPC. (◇) *Endo*-Gly^{0a} channels; (◆) *des*-Val^{1}gC channels. DPhPC/*n*-decane, 200 mV, 1 M NaCl, 25°C.

Gramicidin Channels of Different Lengths

If the LPLs enhance gramicidin channel formation by decreasing the energetic cost of adjusting the hydrophobic thickness of the membrane to the (shorter) hydrophobic length of a gramicidin channel, the gramicidin channel length should be a determinant of the effect of the LPLs. To examine whether this was the case,

experiments were done on gramicidin analogues that were either chain-extended or -shortened. In these experiments, two analogues of gramicidin C (in which Trp[11] has been replaced by Tyr) were used: *des*-Val[1]gC, which is 14 amino acids long and *endo*-Gly[0a]-gC which is 16 amino acids long. Both analogues form conducting channels that are structurally similar to gramicidin A channels (Durkin et al., 1993) and shorter than the equilibrium thickness of the hydrophobic bilayer core. *des*-Val[1]-gC is two residues shorter than *endo*-Gly[0a]-gC, and the hydrophobic length of the *des*-Val[1]-gC channel will be ~0.32 nm less than that of the *endo*-Gly[0a]-gC channel (the length of a $\beta^{6.3}$-helical monomer varies by ~0.08 nm per residue). The effect of LPC on the average duration of these channels is shown in Fig. 8. 2 μM LPC increases τ of *des*-Val[1]-gC channels sixfold, whereas τ of *endo*-Gly[0a]-gC is increased only twofold. The effect of LPC is larger on the shorter channel.

DISCUSSION

Lysophospholipids have profound effects on the behavior of gramicidin channels in planar lipid bilayers. The channel activity and average duration are increased, the single-channel conduction is decreased. The relative effect of LPLs on channel activity and average duration varies with the size of their head group: LPC and LPI are markedly more potent than LPE and LPS. The increases in channel activity are due primarily to changes in the channel appearance rate, and not to an increased adsorption of gramicidin to the bilayer. The head group charge is not important for the changes in channel activity and duration. The average durations of channels formed by the chain-shortened or chain-extended gramicidin analogues *des*-[Val[1]]-gC and *endo*-Gly[0a]-gC are also prolonged by LPC. The effect is larger for the shorter channels, which suggests that LPC alters the membrane deformation energy. Moreover, LPC does not alter membrane thickness or the surface tension of monolayers at the air/electrolyte interface. Addition of the bilayer-forming GMO to DPhPC/*n*-decane bilayers does not increase the duration of gA channels, which further highlights the importance of the size of the polar head groups relative to the cross-sectional area of the acyl chains. Together, these results show that LPLs modulate the function (stability) of gramicidin channels by changing the energetic cost of the membrane deformation associated with channel formation, and that the change in energetic cost can be related to the molecular shape of the LPLs.

Membrane Concentrations of Lysophospholipids

The effects of an LPL will vary as a function of its concentration (molar ratio) in the membrane, which is not known (but can be estimated, see below). For the present arguments, however, it is sufficient to know whether the relative potencies of the LPLs could result from different membrane adsorption. The adsorption coefficient of a LPL is related to its critical micelle concentration (CMC). At low aqueous LPL concentrations ($[LPL]_a$), the mole fraction of the LPLs in the membrane (x_L) should approximately be equal to $[LPL]_a$/CMC (Nichols and Pagano, 1981). The CMC's of different LPLs can be estimated from published values for other micelle-forming phospholipids. The CMC of 1-lauroyl-2-[4-(4,4-dimethyloxazolidine-*N*-oxyl)valeryl]-*sn*-glycero-PC, -PE, and -PS (1 M NaCl, pH 7), for example, varies by less than 50%

(with the -PC derivative having the highest CMC) (King and Marsh, 1987). For a $C_{16:0}$ acyl chain the CMC's of LPC, LPE, and LPS (in 1 M NaCl) should be in the range of 4–6 μM (cf. Marsh and King, 1986). The membrane concentrations of LPC, LPE, and LPS therefore should be comparable at a given $[LPL]_a$. The higher potency of LPC relative to LPE and LPS therefore does not result from a higher membrane adsorption coefficient for LPC. (In fact, the samples of LPE and LPS used here have a higher content of molecules with 18 carbon acyl chains than the LPC [cf. Table I]. This would tend to give them a lower CMC and a higher adsorption coefficient than LPC.)

Based on the above estimates for the CMCs, we estimate x_L to be 0.1, or higher. In agreement with this estimate, addition of LPC to the membrane-forming solution at a molar ratio of 1/4 (LPC/DPhPC) increases τ almost threefold (see Results), which is similar to the change in τ seen at $[LPC]_a = 2$ μM (Fig. 5).

LPLs can cause membrane breakdown at concentrations above their CMC (cf. Weltzien, 1979). In the single-channel experiments, the membranes indeed became less stable in the presence of 4 μM LPL, indicating that we were working at concentrations close to the CMC. At 2 μM, however, the membranes were stable for hours. More importantly, at neither 2 nor 4 μM did we see the nonspecific conductance increases that were described by Sawyer and Andersen (1989) in the case of PAF. The changes in channel behavior we report here therefore are not due to some nonspecific membrane breakdown.

Energetics of Dimerization

A gramicidin channel is formed when two monomers (M) associate to form the conducting dimer (D) (Bamberg and Läuger, 1973; Veatch, Mathies, Eisenberg, and Stryer, 1975; Cifu, Koeppe, and Andersen, 1992). To a first approximation this can be described as:

$$M + M \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D \qquad (3)$$

where k_1 and k_{-1} denote the channel association and dissociation rate constants respectively (Bamberg and Läuger, 1973; but see Cifu et al., 1992).

The dimerization constant (K_D) is given by:

$$K_D = k_1/k_{-1} = [D]/[M]^2. \qquad (4)$$

The sum of M and D constitutes the total amount of available gA (T) in the membrane:

$$[T] = [M] + 2 \cdot [D] \qquad \text{or} \qquad [M] = [T] - 2 \cdot [D], \qquad (5)$$

and

$$K_D = [D]/([T] - 2 \cdot [D])^2 \qquad (6a)$$

which reduces to

$$K_D \approx [D]/[T]^2 \tag{6b}$$

when $M \gg D$. When the surface density of monomers is much larger than that of dimers, the dimerization constant will be proportional to the number of dimers in the membrane. If this condition is not met, the actual increase in K_D will be larger than estimated using Eq. 6b. A minimum estimate of the effect of LPLs on K_D can thus be obtained from the change in the number of conducting channels (cf. Figs. 6 and 7 *B* in Results). The effect is large for all LPLs, but LPC and LPI are much more potent than LPE and LPS.

The population of monomers directly involved in the monomer ↔ dimer equilibrium could be in equilibrium with a population of monomers (M') that is not directly involved in channel formation (Cifu et al., 1992), and the change in the number of conducting channels could result from a change in the M/M' equilibrium (e.g., a transition from non-β-helical to β-helical monomers) as well as a shift in the M/D equilibrium. But a shift in the M/M' equilibrium would not account for the change in channel duration that is induced by all the LPLs, and probably not for the relation between the size of the LPL head group and the ability to change K_D.

At the highest $[\text{LPL}]_a$ used in these experiments (2 μM), the effect on K_D tends to level off (Fig. 7 *B*), which could result from the fact that all (or almost all) the gramicidin was in the dimeric channel form. The experiments where gramicidin was added through the membrane-forming solution provide insights into this question, because the amount of gramicidin in the membrane should be known. In the absence of LPC, the conductance of the gramicidin-doped membranes was ~400 pS, which corresponds to ~30 channels in the membrane (g = 15 pS). The membrane area is ~2 mm^2, and the cross-sectional area of a phospholipid molecule is ~0.7 nm^2, which means there are ~$3 \cdot 10^{12}$ lipid molecules in each monolayer. The molar ratio of gramicidin to phospholipid is 1:10^7 and the theoretical maximum number of channels in the membrane is ~$3 \cdot 10^5$. If all the gramicidin molecules formed channels when the LPL was added, the conductance could rise by a factor of 10^4. The maximal effect we see is ~20-fold less than this upper limit. If the molar ratio of gramicidin to lipid in the bilayer is similar to that in the membrane-forming solution, the saturation observed at higher LPL concentrations cannot be due to reduction in the monomer density.

The standard free energy of channel formation ΔG° is related to K_D by:

$$K_D = \exp\{-\Delta G^\circ/RT\}, \tag{7}$$

where R is the gas constant and T the temperature in Kelvin. The difference between the standard free energy of the dimer in the absence (ΔG°_C) and in the presence of LPLs (ΔG°_L) is given by:

$$\Delta\Delta G^\circ = \Delta G^\circ_L - \Delta G^\circ_C = -RT \ln\{K_L/K_C\} \tag{8}$$

In Fig. 9 we show how $\Delta\Delta G^\circ$ varies as a function of the LPL concentration. $\Delta\Delta G^\circ$ is negative for all the LPLs: at 2 μM LPC and LPI it is ~ −15 kJ/mol; at the same concentration of LPE and LPS, $\Delta\Delta G^\circ$ is only ~ −10 kJ/mol.

The Effects of Lysophospholipids Are Not Due to Specific Interactions with the Channels

In spite of their different polar head groups, the LPLs all decrease ΔG° of gA channels. Furthermore, LPC increases τ of both *des*-Val[1]-gC and *endo*-Gly[0a]-gC channels. The LPLs therefore do not seem to exert their effect through specific interaction between the LPLs and gramicidin. This is in accordance with earlier results from this laboratory, which showed that micelle-forming compounds (detergents) of widely different structure promote gramicidin channel formation and increase τ (Sawyer et al., 1989). The changes in K_D (ΔG°) arise because the dimer has been stabilized by the presence of the LPLs (or detergents) in the membrane. This stabilization could result because LPLs or other detergents bind directly to the channels or because these compounds somehow alter the ability of the membrane to

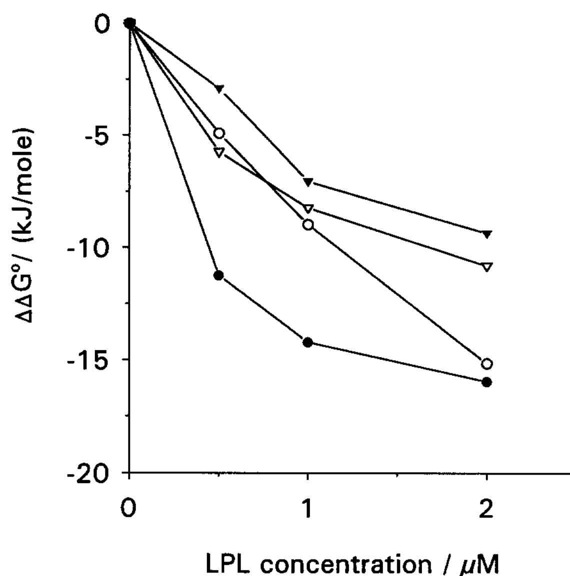

FIGURE 9. The difference in standard free energy ($\Delta\Delta G^{\circ}$) of a gramicidin channel in the absence and presence of LPLs. $\Delta\Delta G^{\circ} = -RT \ln\{K_L/K_C\}$, where R is the gas constant and T the temperature in Kelvin. (○) LPC; (●) LPI; (▽) LPE; (▼) LPS. DPhPC/*n*-decane, 1 M NaCl, 25°C.

adjust to the formation of a membrane-spanning channel. In the absence of a specific interaction, we conclude that the ability of LPLs to alter gramicidin channel function (stability) results from altered membrane properties.

Membrane Fluidity

Membrane-protein interactions are often interpreted in terms of changes in membrane fluidity (e.g., Aloia, Curtain, and Gordon, 1988). At high concentrations, for example, LPLs can decrease the acyl chain order parameter of lipid membranes (Fink and Gross, 1984). This is usually interpreted to signify that the fluidity of the membrane interior is increased, and the effects of LPLs (and other amphipathic lipid metabolites) on membrane protein function have traditionally been ascribed to their effect on membrane fluidity (e.g., DaTorre, Creer, Pogwizd, and Corr, 1991). The

causal relationship between a change in membrane fluidity and the change in the function of embedded proteins has never been clear, however (e.g., Lee, 1991). Furthermore, while fluidity may be important for determining the rate at which a protein can change conformation, the equilibrium distribution between different states, will be determined only by the difference in free energy between these states (cf. Eisenberg and Crothers, 1979), which will not be affected by changes in fluidity per se.

The results obtained in this study cannot be explained in terms of fluidity changes. An increase in the fluidity would be expected to increase the rate constant for channel formation as well as for channel dissociation, but have no effect on K_D. (The rate constants could be almost unaffected by fluidity changes if the reactions were determined exclusively by the intrinsic dynamics of the channel forming molecules.) While the rate constant for channel formation indeed is increased, the dissociation rate constant is decreased, a finding that is in distinct conflict with what would be expected from an increased membrane fluidity.

Gramicidin Channel Formation and Membrane Deformation

LPLs do not interact specifically with gramicidin channels, and changes in membrane fluidity can likewise be ruled out as a cause of the increased channel stability. One is thus, by a process of exclusion, left with the possibility that the increased channel activity results from changes in the mechanical properties of the host bilayer. We will examine this notion in some detail below.

The length of the gramicidin channel's hydrophobic exterior is ~2.2 nm (Elliott et al., 1983; Huang, 1986), which should be compared with the equilibrium thickness of the hydrophobic core of a decane-containing DPhPC membrane, ~4.7 nm (see Results), and the hydrophobic thickness of a hydrocarbon-free di-$C_{16:0}$-PC bilayer in the liquid-crystalline phase, ~2.6 nm (Lewis and Engelman, 1983). According to either measure, the channel length will be less than that of the unperturbed host bilayer. But, at the membrane-channel interface, the thickness of the membrane's hydrophobic core will tend to match the length of the channel's hydrophobic exterior surface (Hendry et al., 1978; Elliott et al., 1983; Mouritsen and Bloom, 1984), which means that channel formation will be associated with a deformation of the surrounding membrane (Elliott et al., 1983; Hladky and Haydon, 1984; Huang, 1986; Helfrich and Jakobsson, 1990). The energetic cost of deforming the membrane will contribute to the overall standard free energy of the channel.

The energetics of the membrane deformation associated with gramicidin channel formation has been analyzed by Huang (1986) and Helfrich and Jakobsson (1990) using a continuum liquid crystal analysis following Helfrich (1973). In the analysis, the channel is considered to be much stiffer than the membrane, such that the channel length is constant. (The length of a gramicidin channel is independent of the thickness of the membrane in which it is embedded [Katsaras, Prosser, Stinson, and Davis, 1992]). Fig. 10 shows, schematically, a gramicidin channel incorporated in a lipid bilayer in the absence (Fig. 10 *A*) and presence (Fig. 10 *B*) of LPL.

The free energy of deforming a bilayer (ΔG_{def}) can be separated into three terms (Huang, 1986; Helfrich and Jakobsson, 1990; see Fig. 10 *C*): the bilayer compression energy due to the change in membrane thickness (compression of the lipid molecules

in a direction perpendicular to the membrane-solution interface), in solvent-containing membranes, one must also consider the cost associated with removing the hydrocarbon from the membrane core; the surface energy due to the increase in the area of the membrane/solution interface, which has a finite surface tension; and the monolayer splay energy due to the changes in the cross-sectional area available for the acyl chains along their length (from the interface to the terminal methyl group). The strict separation of these contributions to the membrane deformation energy may be problematic in cases where the radii of curvature are comparable to the linear dimensions of the lipid molecules (cf. de Gennes and Prost, 1993), as is the case here. Nevertheless, the liquid-crystal analysis provides a coherent framework to discuss the forces involved in the membrane deformation.

Surface tension. Traditionally, the surface tension has been viewed as a major determinant of the energetics of gramicidin channel formation. Assuming a square-well deformation of the lipid membrane around a gramicidin channel, Hendry et al.

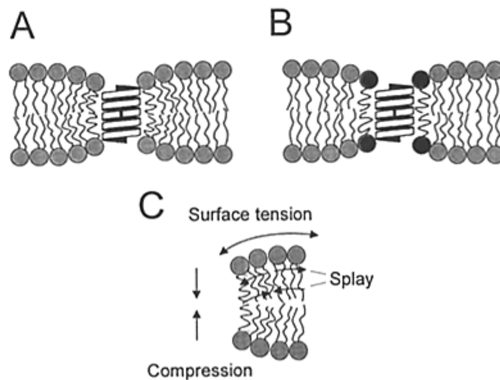

FIGURE 10. (*A*) Schematic illustration of a gramicidin channel in a phospholipid bilayer. The hydrophobic exterior surface of the channel is shorter than the equilibrium hydrophobic thickness of the bilayer core, and channel formation will be associated with a deformation of the bilayer. (*B*) The presence of LPLs in the dimple around the channel will tend to decrease the membrane deformation energy. (*C*) The bilayer deformation energy can be separated into three different components, due to: compression, surface tension, and splay (Huang, 1986; Helfrich and Jakobsson, 1990).

(1978) expressed the channel dimerization constant as a function of the membrane surface tension as:

$$K_D \approx K_o \exp\{-2\pi r\sigma(h - h_o)/kT\}, \quad (9)$$

where K_D is the channel dimerization constant in a membrane of thickness h, K_o the dimerization constant when the membrane thickness is equal to the channel's hydrophobic length h_o, σ the interfacial tension of the membrane-solution interface, and r the radius of the square-well deformation (~0.8 nm). The change in surface tension needed to produce a 500-fold increase in the dimerization constant can be estimated using the relation:

$$K_L/K_C \approx \exp\{-2\pi r(\sigma_L - \sigma_C)(h - h_o)/kT\}, \quad (10)$$

where K_L and K_C, and σ_L and σ_C, denote the dimerization constants, and interfacial tensions, in the presence and absence of LPL, respectively. h_o is ~2.2 nm and r is

~0.8 nm (Elliott et al., 1983); setting $h = 4.7$ nm (the hydrophobic thickness of the DPhPC/n-decane membrane) one finds that $500 \approx \exp\{-2\pi\, 0.8(\sigma_L - \sigma_C)(4.7 - 2.2)/kT\}$, which means that $\sigma_L - \sigma_C \approx -2$ mN/m. If all the hydrocarbon solvent were squeezed out of the membrane immediately adjacent to the channel, however, the appropriate value for h would be closer to that of a $C_{16:0}$ phospholipid bilayer, ~2.6 nm (Lewis and Engelman, 1983), and $\sigma_L - \sigma_C$ would need to change by ~13 mN/m.

There was no effect of 4 μM LPC on the surface tension of DPhPC monolayers at the air/electrolyte interface (see Results). The interfacial tension at the bilayer-solution interface can be estimated from the measured surface tension of the DPhPC monolayer at the air/electrolyte interface (25.9 mN/m), which incorporates a contribution from the electrolyte/hydrocarbon and the hydrocarbon/air interfaces. The former contribution should be close to the surface tension of an air/n-octane interface (21.8 mN/m at 20°C, [Davies and Rideal, 1963]). The monolayer/solution interfacial tension should thus be 25.9–21.8 mN/m or 4.1 mN/m. If the change in K_D resulted solely from a change in the interfacial tension, the LPL's should decrease the interfacial tension by at least 50%! If the interfacial tension was a dominant determinant of K_D, it is difficult to understand why such a dramatic change in dimerization constant could take place without a change in surface tension. The energetic cost of increasing the area of the membrane/solution interface may not be simply related to the macroscopic surface tension, however, in cases where the area increase results from a membrane deformation with a radius of curvature that is comparable to the dimensions of the lipid molecules.

Membrane thickness and channel length. LPLs could exert their effect by altering (decreasing) the membrane thickness (relative to the channel length). But there was no effect of 2 μM LPC on the capacitance (thickness) of the bilayers. When comparing our results with those of Kolb and Bamberg (1977), who examined the effect of membrane thickness on τ, we find that a 30% decrease in thickness would be needed in order to account for the observed increase in τ between 0 and 4 μM LPC (assuming that the cost of deforming a DPhPC/n-decane membrane is comparable to that of deforming the monoglyceride/n-decane membranes used by Kolb and Bamberg, 1977). A thickness change of this magnitude is incompatible with our results.

LPC increases the duration of channels formed by *des*-Val[1]-gC and *endo*-Gly[0a]-gC, and the effect is larger for the shorter *des*-Val[1]-gC channels. The extent of a membrane deformation around a gramicidin channel will be larger for the shorter channel and the magnitude of the membrane deformation energy will be correspondingly increased. As LPLs do not alter the membrane thickness, we conclude that LPLs alter channel duration because of their ability to decrease the membrane deformation energy associated with channel formation.

Association and Dissociation Rate Constants

The average channel duration directly reflects the channel dissociation rate constant ($k_{-1} = 1/\tau$). The relative change in $k_{-1}(k_{-1,L}/k_{-1,C})$ is given by τ_C/τ_L. The results are shown in Fig. 11 *A*. At 2 μM, LPC and LPI decrease this ratio to ~0.3 (0.25 and 0.31, respectively) of the control value, whereas LPE and LPS decrease the ratio only to

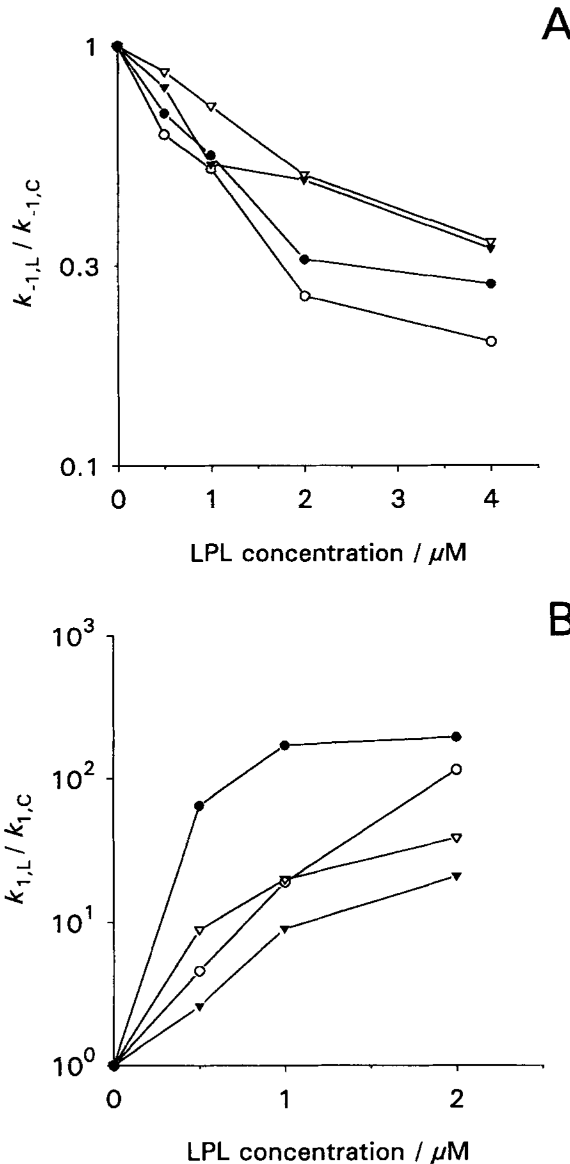

FIGURE 11. (*A*) Dimer dissociation rate constant relative to control as a function of the aqueous LPL concentration. (○) LPC; (●) LPI; (▽) LPE; (▼) LPS. (*B*) Monomer association rate constant relative to control as a function of the aqueous LPL concentration. Symbols as in *A*. DPhPC/*n*-decane, 1 M NaCl, 25°C.

~0.5 (0.49 and 0.48, respectively) of control. Correspondingly, the increase in the association rate constant (k_1) can be expressed as: $k_{1,L}/k_{1,C} = (K_L/K_C)\cdot(k_{-1,L}/k_{-1,C})$. The results are shown in Fig. 11 *B*. At 2 μM, LPC and LPI increase the dimerization rate constant 100-200–fold, whereas LPE and LPS increase it only ~30-fold.

The channel stabilization, as reflected by the increase in K_D, results primarily from an increased channel formation rate constant. This can be rationalized by noting that the deformation energy will be an approximately quadratic function of the depth of the deformation (Mouritsen and Bloom, 1984; Huang, 1986; Durkin et al., 1993):

The association of gramicidin monomers involves a decrease of the hydrophobic bilayer thickness from 2.6 nm (4.7 nm for DPhPC/*n*-decane membranes) down to 2.2 nm, while the dissociation step involves a separation of the monomers (and thus an increase in the bilayer thickness) of only ~0.1 nm (Huang, 1986; Durkin et al., 1993). A change in membrane deformability therefore would be expected to have a larger effect on k_1 than on k_{-1}.

The Molecular Shape of Lysophospholipids and Membrane Deformability

The ability of LPLs to decrease the membrane deformation energy associated with the formation of a gramicidin channels can be explained by considering the molecular shape of these compounds. Bilayer-forming molecules, such as phospholipids, and monoglycerides have a roughly cylindrical shape, as the cross-sectional area of the polar head group at the lipid-solution interface is comparable to that of the acyl chains in the core of the bilayer (Fig. 12 *A*). In the case of LPLs, the cross-sectional area of the polar head group will be greater than that of the single acyl chain, e.g., Carnie et al. (1979). These molecules can therefore be considered to be cone shaped, with the base of the cone at the polar-nonpolar interface, and they

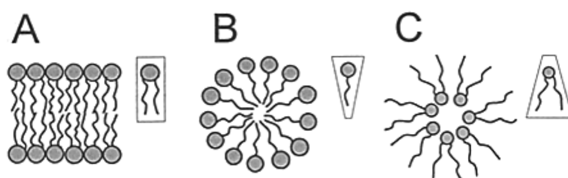

FIGURE 12. Shapes of amphipathic molecules and the structures they form. According to their molecular shapes, lipid and detergent molecules will tend to form either: (*A*) bilayers; (*B*) micelles; or (*C*) H_{II}-structures (Cullis and De Kruijff, 1979).

will tend to stabilize convex surfaces (Fig. 12 *B*). Cone shaped molecules that have the base toward their nonpolar tail will, in contrast, tend to stabilize concave surfaces (Fig. 12 *C*).

More generally, the membrane architecture and the physical properties of the membrane depends on the molecular shape of the molecules in the membrane (e.g., Carnie et al., 1979; Cullis and De Kruijff, 1979). In small unilamellar vesicles, for example, LPC tends to reside in the outer bilayer leaflet (e.g., de Kruijff, van den Besselaar, and van Deenen, 1977; Kumar, Malewicz, and Bauman, 1989). Furthermore, whereas LPC destabilizes planar bilayers (Weltzien, 1979), in small unilamellar vesicles (radius ~26 nm) of diacylPC and LPC the asymmetric incorporation of the cone-shaped LPC into the outer monolayer restricts head group motion of the cylindrically shaped diacylPC molecule, presumably tightening the phospholipid packing in this highly curved monolayer (Kumar et al., 1989).

The distribution of lipid molecules in a bilayer therefore seems to be such as to minimize the mechanical strain in the bilayer. The asymmetrical distribution of LPC molecules in lipid vesicles, for example, results in a decreased bilayer strain, because the presence of these cone-shaped molecules in a monolayer will tend to decrease the

energetic cost of a convex membrane deformation and increase the cost associated with a concave deformation. This provides for a certain specificity in terms of how LPLs can modify the function of membrane proteins. Convex monolayers, for example, will be relative enriched in LPLs.

The formation of a gramicidin channel involves a convex deformation of the two monolayers (Fig. 10 *A*). The presence of cone-shaped lipids in the bilayer will tend to decrease the energetic cost of the membrane deformation and thereby decrease the overall energetic cost of channel formation (Fig. 10 *B*). The general effect of LPLs on the energetics of gramicidin channel formation therefore can be explained by a decreased cost of the membrane deformation associated with channel formation. Moreover, the relative potency of different compounds can be rationalized by considering their generalized molecular shapes. The effect of LPI and LPC on K_D is 10-fold higher than that of LPE and LPS, a result that also can be rationalized by considering the different shapes of these compounds. The acyl chains have comparable cross-sectional areas, and the variation in potency is related to the size of the polar head group. The specific substituents at the phosphorous atom in the LPLs are shown in Fig. 13, their volumes are listed in the legend.

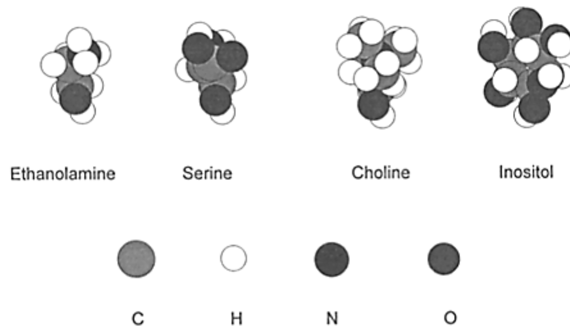

FIGURE 13. The specific substituents at the phosphorous atom. The atoms are identified below the four substituents. The volumes of the choline (0.101 nm^3), ethanolamine (0.063 nm^3), and serine (0.087 nm^3) moieties were calculated from the van der Waals volumes of amino acids (Creighton, 1993) using simple additivity of the substituent volumes and setting the volume of a CH_3 group to 0.02 nm^3. The volume of inositol (0.187 nm^3) was calculated from the partial molar volume of solid inositol (Weast, 1972).

In addition to the molecular volume, one must also consider the hydration of the different head groups. Phosphatidylcholine head groups, for example are much more hydrated than the corresponding phosphatidylethanolamine and phosphatidylserine head groups (Elworthy, 1962; Jendrasiak and Hasty, 1974; Sen and Hui, 1988; Marsh, 1989; Rand and Parsegian, 1989). This hydration will serve to increase the effective size of a phosphatidylcholine head group, and a LPC head group should be markedly larger than those of LPE and LPS. (The hydration of phosphatidylinositol has not been measured but is likely to be small; inositol is not hygroscopic and digalactosyldiacylglyceride, which also has a carbohydrate head group, is poorly hydrated in bilayer phase [Sen and Hui, 1988; Rand and Parsegian, 1989].) Thus, one can account for the relative potencies of the LPLs on K_D based on their molecular shapes.

GMO also has a single acyl chain, but the cross-sectional area of the polar group is comparable to (or less than) that of the acyl chain. In accordance with its cylindrical shape, GMO forms bilayers. If the shape concept is valid, that is if the effect of LPLs

on the gramicidin channel is a consequence of their molecular shape, then GMO should not increase τ. This is indeed the case. In fact, GMO decreases τ slightly, which may be related to GMO's ability to promote the formation of H_{II} phases (Keller et al., 1993).

Single-Channel Conductance

All the LPLs decrease g in a concentration-dependent manner. The basis for this conductance decrease is not clear; but similar conductance changes have been observed with other micelle-forming surfactants (Sawyer et al., 1989), and the effect is independent of the type and concentration of the permeant ion (D. B. Sawyer and O. S. Andersen, unpublished observations). One possibility is that the cone-shaped LPLs allow for an increased density of head groups in the membrane surrounding the channel, which will establish a larger (more positive) interfacial dipole potential (Andersen, 1978) in the vicinity of the channel. A more positive dipole potential will in turn decrease g (Jordan, 1983). That LPI and LPS are less effective in decreasing g is presumably due to the fact that they carry a net negative charge, which will establish a negative surface potential as they adsorp to the membrane (cf. McLaughlin, 1977). This will, in turn, increase the conductance of the cation-selective gramicidin channels (Apell et al., 1979; Szabo and Busath, 1983).

Implications for Integral Membrane Proteins

In addition to their structural role, the lipids of cell membranes regulate the function of the imbedded membrane proteins in an apparently rather nonspecific manner (Devaux and Seigneuret, 1985; Bienvenüe and Marie, 1994). The mechanisms that underlie this regulation remain largely unknown. In recent years the importance of the shape of membrane lipids for the function of membrane proteins has been emphasized (cf. Gruner, 1989, 1991; Gibson and Brown, 1993; Keller et al., 1993). It has also become clear that a liquid-crystalline bilayer is necessary for protein function (e.g., McNamee and Fung, 1988), but that membrane "fluidity" by itself cannot by itself be a (major) determinant of protein function (McNamee and Fung, 1988; Lee, 1991; Zakim, Kavecansky, and Scarlata, 1992). At the present time no clear rule exists to rationalize the effect of lipid composition on ligand binding to or enzyme activity of integral membrane proteins (Bienvenüe and Marie, 1994).

LPLs alter the stability of gramicidin channels by changing the mechanical properties of the host lipid bilayer. Similar changes in bilayer deformation energy may constitute a general mechanism whereby the host bilayer can influence the function of integral membrane proteins, because quaternary conformational changes in the membrane-spanning part of a protein will alter the hydrophobic interface between the protein and the surrounding bilayer. Such changes have been described for gap junction channels (Unwin and Ennis, 1984) and the nicotinic acetylcholine receptor (Unwin, Toyoshima, and Kubalek, 1988), and have been related to different functional states of the proteins. In gap junction channels, for example, the transition between the closed and open channel states is associated with a 7.5° change in the tilt of each connexin relative to the plane of the membrane (Unwin and Ennis, 1984). Such a change in the membrane spanning part of the protein would change the channels hydrophobic length ($\sim$3 nm) by $3 \cdot (1 - \cos\{7.5°\})$ or $\sim$0.03 nm. If this

change occurred with no change in bilayer thickness, it would mean that a hydrophobic surface with an area of $\sim 2\pi \cdot 3 \cdot 0.03$ nm^2 or ~ 0.6 nm^2 would be exposed to the aqueous surrounding (the external radius of a gap junction channel is ~3 nm [Unwin and Ennis, 1984]). The energetic cost of exposing a hydrophobic surface to an aqueous solution is ~20 kJ/nm^2 (Sharp, Nicholls, Fine, and Honig, 1991), and the energetic cost of this exposure would be ~12 kJ (or ~5 kT at 25°C), which will cause the surrounding bilayer to adjust to the change in protein conformation. The energetic cost of the membrane deformation will contribute to the free energy difference between the different protein conformations.

The important point is that the coupling between integral membrane proteins and their surrounding lipid bilayers can give rise to substantial interaction energies, which will contribute to the overall free energy difference between different states of the protein. Changes in membrane lipid composition, or pharmacologically induced alterations in membrane properties, can alter membrane protein function by altering the membrane deformation energy. One should also note that maneuvers that alter a bilayer's fluidity almost invariably will alter the bilayer's deformation energy; but with no fixed relation between the change in fluidity and the change in deformation energy. In conclusion, the membrane deformation energy is important for membrane protein function; this may serve as a general framework to rationalize the importance of the lipid bilayer for membrane protein function.

We finally note that activation of phospholipase A_2 in ischemia (Sobel, Corr, Robison, Goldstein, Witkowski, and Klein, 1978; Hazen and Gross, 1992) leads to the production of (nonbilayer forming) LPLs and free fatty acids. The pathophysiological importance of the ensuing lipid metabolite accumulation has long been recognized (e.g., Katz and Messineo, 1981), and the modification of membrane protein function by LPLs has been implicated in the electrophysiological disturbances that occur early in coronary ischemia (Sobel et al., 1978). Similar changes in neuronal membranes occur early in cerebral ischemia (Katsura, de Turco, Folbergrová, and Siesjö, 1993) and may be responsible for some of the electrophysiological disturbances that occur during ischemia (Hansen, 1985). LPC, for example, alters the gating of voltage-dependent sodium channels (Burnashev et al., 1989); and LPLs inhibit the activity of ATP-sensitive potassium channels (Eddlestone and Ciani, 1991), with a relative potency of LPC, LPE, LPI, and LPS that parallels their effect on gramicidin channels.

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