J. Membrane Biol. 145, 13–19 (1995)

The Journal of
Membrane
Biology

Lipid-Ion Channel Interactions: Increasing Phospholipid Headgroup Size but not Ordering Acyl Chains Alters Reconstituted Channel Behavior

H.M. Chang, R. Reitstetter, R. Gruener
University of Arizona College of Medicine, Department of Physiology, Tucson, Arizona 85724

Received: 2 September 1994/Revised: 1 December 1994

Abstract. We have recently shown (Chang et al., 1995) that lipid-channel interactions, exemplified by the effects of cholesterol on the calcium-activated potassium (BK) channel, profoundly affect channel properties. The present study further explores such interactions by monitoring changes in BK channel behavior after reconstitution into bilayers where the size of phospholipid (PL) headgroups is increased and where the freedom of motion (inverse order) of fatty acid chains is incremented. Increasing the PL headgroup cross-sectional area, from that of *N*-meth-DOPE to that of DOPC (an increase from ca. 60 to 70 $Å^2$), is associated with a doubling of the channel mean opentime. Channel conductance, however, was unaffected. Increasing the order of the fatty acid chains, from that of DOPE to POPE and to that of DEPE, had no significant effect on channel properties (at 22°C). We interpret the changes reported here to reflect lipid-protein interactions through the induction of structural stress related to the headgroup structures of phospholipids.

Key words: Lipid-protein interactions — Elastic stress — Curvature stress — Reconstituted potassium channel

Introduction

Evidence is mounting to implicate lipids as functional modulators of membrane integral proteins, such as ion channels, through lipid-protein interactions (Fong & McNamee, 1986; Gibson & Brown, 1993; Keller et al., 1993; Chang et al., 1995; *see* review by McElhaney, 1989). A better understanding of such interactions may be gained by examining how the structure of lipids (different packing order, different headgroup size, or altered acyl chain structure) contributes to physical forces generated in lipid membranes. (*See* for example, Gibson & Brown, 1993; Keller et al., 1993; Chang et al., 1995.) In this paper, we apply the structural stress hypothesis which relates stress forces in bilayers to the modulation of membrane integral protein function. For example, Keller et al. (1993) showed that the probability of finding the alamethicin channel in the open state increases dramatically when it is incorporated into a bilayer made of dioleoylphosphatidylethanolamine (DOPE) rather than of dioleoylphosphatidylcholine (DOPC). In a different system, Gibson & Brown (1993) reported that the M_{II}/M_I ratio of rhodopsin decreased in vesicles where the phosphatidylcholine (PC) concentration was raised (the M_I to M_{II} transition is one step in the activation of rhodopsin). These authors suggest that the change may be related to the physical properties (elastic or curvature stress) of the bilayer. Replacing the ethanolamine headgroup in phosphatidylethanolamine (PE) with a choline moiety (in PC) results in an increase in the effective cross-sectional area of the headgroup because the choline moiety is bulkier and hydrates more fully (Rand et al., 1992). As the phospholipid (PL) headgroup size is increased, leaving the acyl chains unaltered, the spontaneous radius of curvature of the lipid is reduced, resulting in a reduced curvature stress in planar bilayers (Gruner, 1992). The acyl chains of PLs also affect the curvature stress of the bilayer. For example, as the order of fatty acid chains is decreased, by adding unsaturated bonds (e.g., from 18:0 to 18:1Δ^9c), the diameter of the hexagonal cylinder array of lipids is decreased (Marsh, 1990*a*). This causes a decrease in the spontaneous radius of curvature and a consequent increase in the curvature stress when such lipids are forced into a planar bilayer configuration (Gruner et al., 1988; Tate et al., 1992). Tight packing of PLs produces lateral elastic repulsion forces among the lipid molecules, and this repulsion produces lateral elastic

Correspondence to: R. Gruener

stress in bilayers (Evans & Needham, 1987; Needham & Nunn, 1990). This stress force increases when the lipid headgroup cross-sectional area is reduced (Rand & Parsegian, 1992), when the temperature is lowered (i.e., ordering the fatty acid chains; Needham & Evans, 1988) or when cholesterol is inserted into bilayers (Needham & Nunn, 1990). We have recently shown that the mean opentime and conductance, of the BK channel, decreased markedly as the concentration of cholesterol in bilayers was increased (Chang et al., 1995). Experimental evidence also shows that the phase status of lipid membranes contributes to the regulation of integral protein function. For example, the activities of the glucose transporter (Whitesell et al., 1989) and the Na/K-ATPase (Chong, Fortess & Jameson, 1985) decreased as the temperature was lowered below the phase transition temperatures of the membrane lipids surrounding these enzymes. The authors suggest that the reduction in enzyme activity may be due to a transition from the liquid-crystalline to the gel phase of the lipids. This transition results in an increase in the order of the lipids thereby possibly restricting conformational changes associated with enzyme activation.

In the present study, we examine how increasing the size of the PL headgroups and increasing the order of acyl chains of bilayer lipids, into which the channel is reconstituted, affect its functional properties. Our results are consistent with the hypothesis relating increased structural stress in the bilayer with a reduction in the ability of the BK channel to remain in the open state as a result of this force "squeezing" the channel into the closed state.

Materials and Methods

Lipids were purchased from Avanti Polar Lipids (Alabaster, AL) and decane from Sigma (St. Louis, MO). The BK channel, from rat brain, was reconstituted into a set of planar lipid bilayers made from phospholipids with different headgroups, keeping the fatty acid chains the same. In a separate set of experiments, bilayers were made from PL with different fatty acid chains, keeping the headgroups the same. Rat brain P3 vesicles were prepared according to Krueger, Worley & French (1983) with slight modifications (Chang et al., 1995). Phospholipids were prepared as single species, dissolved in decane, at concentrations of 20–30 mg/ml.

To investigate the effects of the cross-sectional areas of PLs, we reconstituted the BK channel into bilayers made of the following lipids whose headgroup dimensions increase in the order: mono-methylated dioleoylphosphatidylethanolamine (*N*-meth-DOPE) (62 $Å^2$) < DOPE (65 $Å^2$) < DOPC (70 $Å^2$; data from Gruner et al., 1988). The fatty acid chains of these three PLs have the same composition and structure (*see below*). To investigate the effects of fatty acid chains on channel properties, PEs with different fatty acid chains were chosen because channel incorporation into such bilayers was found to be more frequent than into bilayers made with PC. DOPE has two oleic acid chains, consisting of an 18-carbon backbone with a cis double bond at position Δ9 ($18:1\Delta^9c$). 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) has one palmitic acid, which consists of a saturated 16-carbon backbone (16:0), and one oleic acid chain. Finally, dielaidoylphosphatidylethanolamine (DEPE) has two elaidic acid chains, composed of an 18-carbon backbone with a trans double bond at position Δ9 ($18:1\Delta^9t$; Marsh, 1990*b*). The major phase transition temperatures (T_c) of the PLs have the following order: DOPE (−16°C) < POPE (+25°C) < DEPE (+35°C) (Lewis et al., 1989, and Marsh, 1990*b*). Thus, the ordered structure of the fatty acid chains (at 22–23°C) may be summarized to be: DOPE < POPE < DEPE. We were concerned about the presence of decane, as a constant contaminant in all experiments. However, its contribution to our results is likely to be small based on previous observations (Labarca, Coronado & Miller, 1980) which show that decane does not significantly affect the conductance of the voltage-dependent K^+ channel reconstituted from sarcoplasmic reticulum. Hexadecane and squalane also have been used by others, as solvents, and they were shown to have no effect on reconstituted alamethicin channels (Keller et al., 1993). Unfortunately, no data are available on the effect of decane on structural aspects of the lipids we used in this study.

Channel incorporation procedures, data acquisition and analysis were as previously described (Chang et al., 1995). Briefly, vesicles from rat brain plasmalemmal fraction were prepared by differential centrifugation and subsequently fused to planar bilayers. The ease of channel incorporation depended on bilayer composition. In general, incorporation into all PE bilayers was easier and faster than into DOPC bilayers. In PE bilayers, incorporation was typically achieved within few minutes after "brushing" the P3 membrane preparation across the bilayer from the trans side chamber. For DOPC bilayers, often a few hours of repetitive trials were needed. Single channel activity was recorded with a patch-clamp amplifier (Axopatch 1A amplifier; Axon Instruments, Foster City, CA). The solution in the trans recording chamber had the following composition (in mM): KCl = 100, $CaCl_2$ = 0.1, HEPES = 10, pH 7.4. The cis solution had the same composition except that KCl = 300. Experiments were carried out at 22.5 ± 0.5°C. The trans side was grounded. Signals were filtered at 3 KHz before being digitized at a sampling rate of 10 KHz. Results from bilayers containing only a single channel were pooled for statistical analysis. Single channel conductance was calculated from the slope of the current-voltage (*I–V*) relationship. The Student *t*-test was used for statistical testing of difference (significance was defined at $P < 0.05$).

ABBREVIATIONS

N-meth-DOPE,	mono-methylated dioleoylphosphatidylethanolamine
DOPC,	dioleoylphosphatidylcholine
DOPE,	dioleoylphosphatidylethanolamine
POPE,	1-palmitoyl-2-oleoyl-phosphatidylethanolamine
DEPE,	dielaidoylphosphatidylethanolamine

Results

Effects of Headgroup Size

The BK channel was reconstituted into bilayers made of *N*-meth-DOPE, DOPE and DOPC, respectively, where the nominal headgroup cross-sectional areas are 62, 65 and 70 $Å^2$. Figure 1 shows typical records of BK channel activity under these conditions. When incorporated into bilayers made of *N*-meth-DOPE (the smallest headgroup size; Fig. 1*A*), the mean opentime (t_o) was rela-

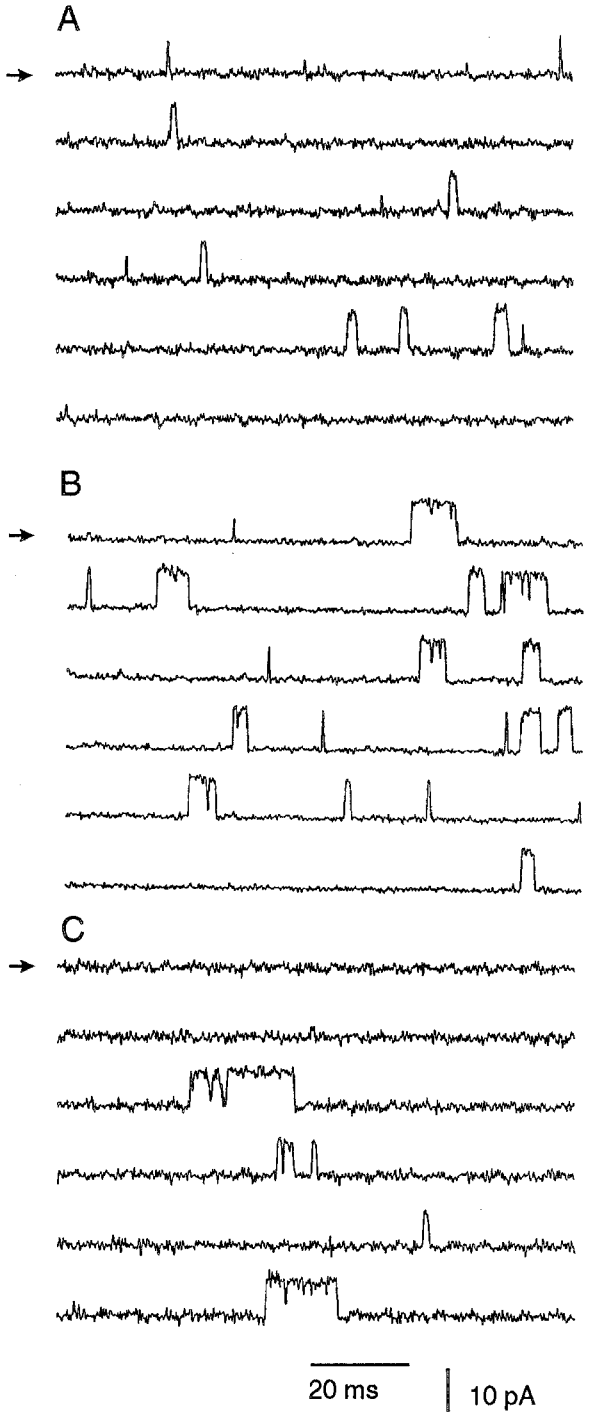

Fig. 1. Activity of single BK channels after reconstitution into bilayers composed of *N*-Meth-DOPE (*A*); DOPE (*B*); and DOPC (*C*). Results are presented in increasing order of headgroup cross-sectional area (*see* Materials and Methods for details). The holding potential (E_h) was +10 mV in all cases. Arrows indicate the closed state of the channel. Note the gradual increase in channel opentime durations.

tively short at 1.3 ± 0.8 msec (mean ± SEM; 288 events). The probability of the channel being in the open state (P_o) was 0.01. The mean current amplitude, in this experiment, was 10.2 ± 0.1 pA. Some open events were too short to be fully resolved and are seen as distorted low amplitude fluctuations. Such events (shorter than 1 msec) were not included in the amplitude statistics in order to minimize artifacts due to bandwidth limitations. When the headgroup size was slightly larger (DOPE at 65 Å^2; Fig. 1*B*), the mean opentime was intermediate at t_o = 2.9 ± 0.1 msec (126 events), and opening events were more frequent (P_o = 0.04). The current amplitude was 9.95 ± 0.02 pA. In the DOPC bilayer, which has the largest cross-sectional area, the mean opentime was the longest at t_o = 3.7 ± 0.5 msec (60 events), and P_o = 0.01. The current amplitude was 9.32 ± 0.08 pA. The opentime histograms for these channels are shown in Fig. 2. Each histogram was best fit with a double exponential decay function. The time constants (τ) for the channel in the *N*-meth-DOPE bilayer (A) were τ_1 = 0.6 msec (59% of all events) and τ_2 = 1.4 msec (41%). In DOPE bilayer (B), τ_1 = 0.4 msec (58%) and τ_2 = 2.6 msec (42%); and in the DOPC bilayer (panel C), τ_1 = 0.6 ms (43%) with τ_2 = 9.8 ms (57%). A double exponential fit of opentime histograms, for the BK channel, also has been reported after reconstitution into other bilayers (Toro, Vaca & Stefani, 1991and Reinhart et al., 1991; Chang et al., 1995). Table 1 summarizes the effects of headgroup size on channel properties from a number of experiments. As the headgroup size increased, the channel mean opentime increased in a monotonic manner. There are no significant changes in τ_1, but the change in τ_2 follows a similar trend to that seen with t_o values. The apparent differences in the values of the time constants (values of Fig. 2 *vs.* Table 1) are due, at least in part, to curve fitting algorithms (Colquhoun & Sigworth, 1983). The open probability shows the highest value in DOPE but the values, among the three different PLs, are not statistically different from each other. Channel conductance did not change.

Effects of Fatty Acid Chain Structure

The BK channel was reconstituted into bilayers, of increasing fatty acid acyl chain order, made of DOPE, POPE, and DEPE respectively (*see* Materials and Methods) in order to determine whether the structure of the bilayer, as influenced by the order of the acyl chains had any effect on channel properties. Typical records of channel activity are shown in Fig. 3. In the DOPE bilayer (Fig. 3*A*), the mean opentime duration was 2.8 msec and is very similar to those in POPE (2.7 msec) and in DEPE (2.8 msec; *B* and *C*, respectively). Table 2 summarizes channel properties after incorporation into such bilayers. The data show that channel mean opentimes (t_o) are not affected by the order of the fatty acids examined here. The open probability of the channel (P_o) and channel conductance (g) also show no significant differences under these conditions.

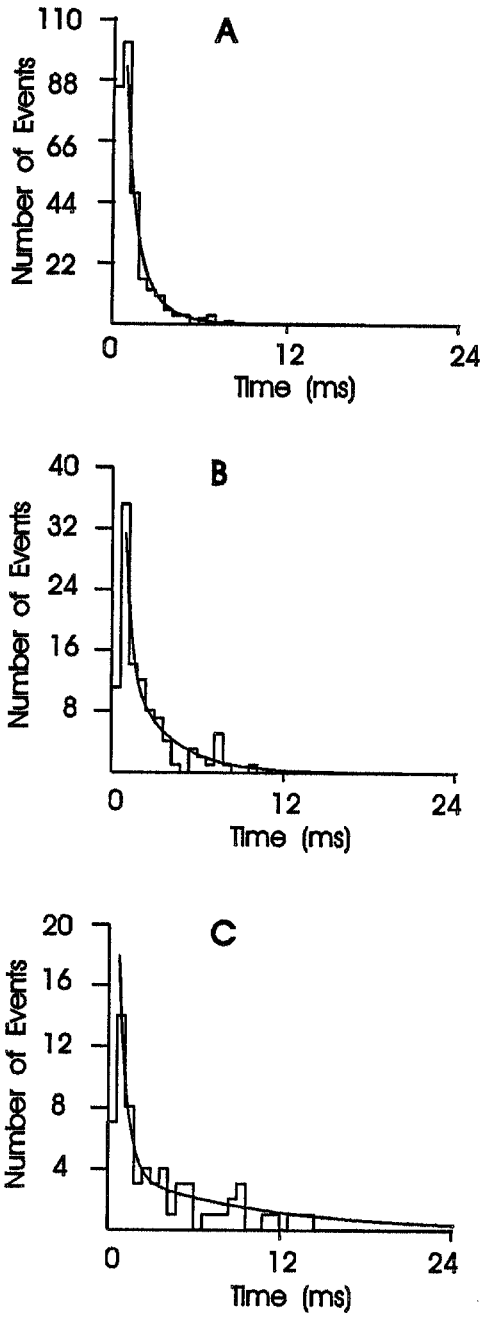

Fig. 2. Opentime histograms of BK channels reconstituted into lipid bilayers with increasing headgroup size (data from the same experiments shown in Fig. 1). The opentime histograms were best fit with double exponential decaying functions. Note the gradual increase in opentime durations. Quantitative comparisons are shown in Table 1.

Discussion

We tested whether the PL headgroup size (with constant acyl chain structure) and the order of acyl chains (with constant headgroup size) affect the behavior of the BK channel incorporated into bilayers made under these conditions. We noted at the outset (*see* Materials and Methods) differences in the ease of channel incorporation depending on the composition of the bilayers. Channel incorporation was easy, compared to that achieved with PE/phosphatidylserine (PS) bilayers (Chang et al., 1995), when reconstituted into PE and was more difficult (required more time and a larger number of applications of the protein fraction to the bilayer) in PC membranes. These differences may relate to the bilayer structure. First, it has been suggested that the tendency of lipids to form hexagonal structure is important in mediating membrane fusion; DOPC does not form hexagonal structures as easily as PEs (Seddon, 1990). Second, the greater hydration of the PC headgroup may reduce the probability of contact between the bilayer and the membrane vesicles containing the BK channel; therefore, the fusion rate of the vesicle with the bilayer may be reduced (Rand & Parsegian, 1992).

On the basis of our previous observations (Chang et al., 1995), we expected to see progressive changes in channel properties as the lateral stress in the bilayer was increased either because of the presence of smaller PL headgroup size or because of a decrease in the order within the PL acyl chains. Indeed, we report here that as the PL headgroup size increases, the channel mean opentime also increases. These results can be explained on the basis of the generation of progressively smaller structural stresses which are likely to develop as the PL headgroup size is increased (Rand & Parsegian, 1992). We have recently shown (Chang et al., 1995) that considerations of structural stress provides a conceptual explanation for the effects of cholesterol on the BK channel. Specifically, as the stress force decreases it imparts less of a reactive force to the opened channel thereby resulting in the tendency of the channel to remain in the open state for longer times. The contribution of the lateral elastic stress, one component of the total stress, can be expected to diminish as the PL headgroup size increases because the modulus of compressibility (K) is lower (Chang et al., 1995; Eq. 6) for lipids with larger headgroups than those with smaller ones (Rand & Parsegian, 1992). (The cross-sectional areas of the lipid headgroups used in this study are: *N*-meth-DOPE 62 $Å^2$, DOPE 65 $Å^2$, and DOPC 70 $Å^2$; Gruner et al., 1988.) In addition to the lateral elastic stress, parallel to the plane of the membrane, another force may be generated from the curvature stress characterized by the spontaneous radius of curvature of the lipids (Gruner, 1992). The increase in free energy resulting from the intrinsic curvature stress, another component of the total structural stress, is inversely proportional to the spontaneous radius of curvature of the lipid (Helfrich, 1973; Gruner, 1985). The spontaneous radius of curvature is approximately equal to the radius of the water column in the lipid hexagonal II [H_{II}] phase (Keller et al., 1993). A planar bilayer made of lipids with large headgroup (e.g., DOPC) will have less curvature-dependent structural stress than a bi-

Table 1. Effects of lipid headgroups on channel properties

	N-meth-DOPE	DOPE	DOPC
Headgroup area (Å^2)‡	62	65	70
t_o (msec)	1.6 ± 0.1	2.7 ± 0.2*	3.5 ± 0.7*
P_o (% of total time)	1.2 ± 0.3	3.5 ± 1.3	1 ± 1
τ_1 (msec)	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.1
τ_2 (msec)	1.6 ± 0.2	3.3 ± 0.3*	5.7 ± 2.1
Conductance (pS)	271 ± 2	277 ± 6	269 ± 7
n	3	6	3
m (M)	260–670 (1200)	100–600 (1800)	60–300 (600)

E_h = +10 mV; *t* = 22.5°C, data shown as mean ± SEM; *n* = number of experiments.
* = significantly different from N-meth-DOPE, *t*-test $P < 0.05$, ‡data from Gruner et al. (1988).
m = Range of number of open events in each data set; M = total number of open events in each lipid condition.

Table 2. Effects of lipid chain structure on channel properties

	DOPE	POPE	DEPE
t_o (msec)	2.7 ± 0.2	2.9 ± 0.7	2.8 ± 0.6
P_o (% of total time)	3.5 ± 1.3	2.5 ± 1.2	4.8 ± 2.8
τ_1 (msec)	0.6 ± 0.2	0.9 ± 0.1	0.8 ± 0.3
τ_2 (msec)	3.3 ± 0.3	3.7 ± 0.9	3.2 ± 0.5
Conductance (pS)	277 ± 6	253 ± 17	266 ± 20
n	6	12	3
m (M)	100–600 (1800)	110–820 (4600)	130–780 (1100)

E_h = $^+$10 mV, t = 22.5°C, data presented as mean ± SEM; *n* = number of experiments.
m = range of number of open events in each data set; M = total number of open events in each lipid condition; no significant differences were observed.

layer made with small headgroup lipids (e.g., DOPE). The rank order of the spontaneous radius of curvature is: 31 Å for DOPE, 38 Å for *N*-meth-DOPE (Rand et al., 1990), and >200Å for DOPC (Keller et al., 1993). Qualitatively, the increased channel mean opentime in DOPC is consistent with the hypothesis that reduced curvature stress causes the channel to stay in the open state longer. However, the spontaneous radius of curvature for *N*-meth-DOPE and DOPE does not exactly follow the same rank order of headgroup sizes. The underlying causes for this discrepancy are not understood (Gruner et al., 1988).

The open probability data we report here show considerable variations. This has been reported previously for the BK channel (Methfessel & Boheim, 1982; Moczydłowski & Latorre, 1983) but the reasons for this finding are unknown. Also, in contrast to the consistent relationship of the mean opentimes with the physical parameters of the bilayers (as determined by the PL headgroup size), the open probability (P_o) values do not follow a consistent correlation with the size of headgroups. One possible explanation for this may be the fact that the probability of opening of the BK channels depends on the binding of Ca^{2+}. We cannot rule out a possible effect of changes in the lipid environment on the capacity of the channel to bind calcium (Starling, East & Lee, 1993; Trave et al., 1994); such an alteration would alter channel kinetics. Our data show further that channel conductance was not affected by the size of the PL headgroup. A similar finding was reported for the conductance of the alamethicin channel when incorporated into bilayers made with DOPE or DOPC (Keller et al., 1993).

When the BK channel was reconstituted into bilayers with increasing acyl chain order (DOPE, POPE and DEPE, respectively), we observed no significant changes in channel properties. Our experiments were carried out at ~22°C where DOPE ($T_c = -16$°C) would be above and POPE (T_c = 25°C) and DEPE (T_c = 35°C) would be below their transition temperatures. Therefore, at room temperature, the expected order of the fatty acid chains may be taken as: DOPE < POPE < DEPE. The modulus of compressibility K has been shown to be linked to the phase state of the lipid, and therefore to the order of acyl chains. A ~50% increase in K occurs when the lipid is below T_c (Needham & Evans, 1988). Even though there are no exact K values available for the three lipids used in this study, it is reasonable to expect on the basis of increasing order that the rank of the K values will be: DOPE < POPE < DEPE. Since the lateral elastic stress

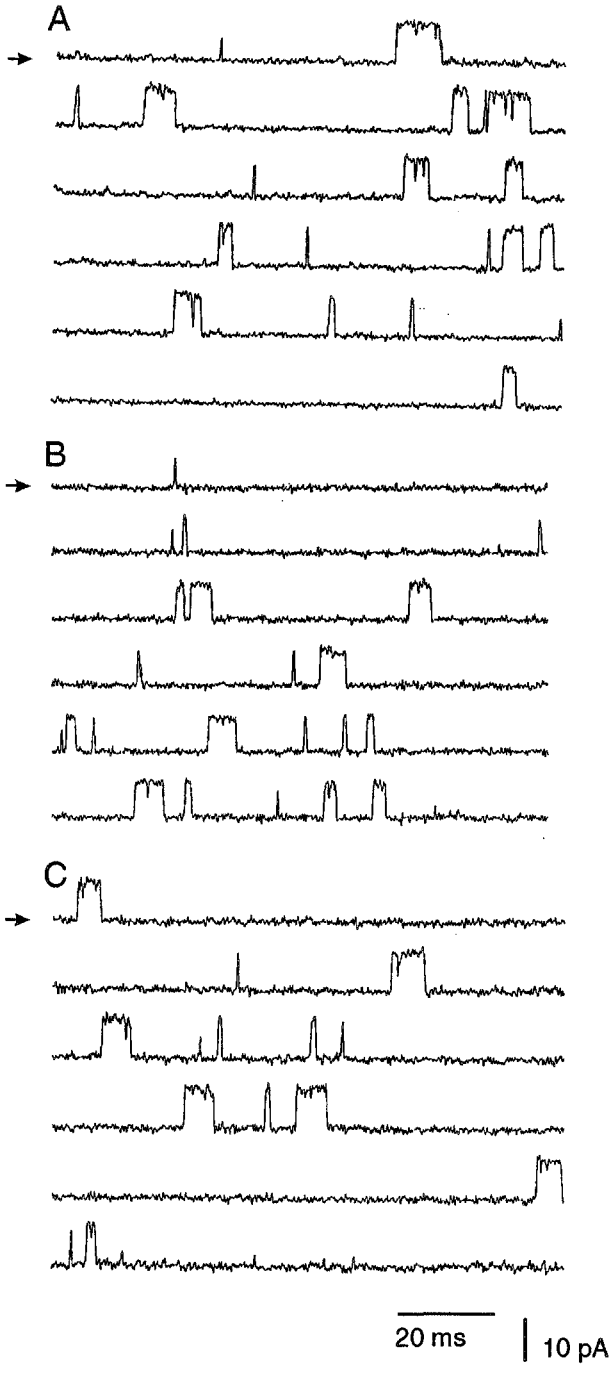

Fig. 3. Records of single BK channel events after reconstitution into bilayers made of DOPE (*A*), POPE (*B*), and DEPE (*C*). Other details are as in Fig. 1.

is proportional to K (Eq. 1), the rank of the lateral elastic stress in the bilayer should follow the same sequence. The curvature stress has also been shown to be dependent on acyl chain composition. Thus, the radius of curvature, in the hexagonal phase, is 25% larger for DEPE than for DOPE (Tate et al., 1992). Therefore, the increased order of fatty acid chains (which results from transforming a cis into a trans double bond) antagonizes the tendency of the lipid to form a hexagonal structure. This increase in spontaneous radius of curvature of the lipid bilayer may result in a reduction of curvature stress in the planar bilayer. It is therefore possible that when the lipid composition is altered from DOPE to DEPE the change in structural stress is likely to be very small. One would then expect little or no effects to take place on the properties of the BK channel even though the order parameter of the lipid is increased. These arguments show that considering the order of fatty acid chains alone is insufficient to predict the effects of lipids on membrane-integral proteins. Possible changes in the overall bilayer thickness did not appear to influence channel properties in our experiments. First, the thickness of the bilayer does not appear to change significantly when the size of headgroup is increased (Marsh, 1990*c*). Second, although there may be an increase in the bilayer thickness when the double bond in the fatty acid chain is changed from cis to trans configuration (i.e., DOPE to DEPE), no changes in channel properties have been observed under these conditions. In the same vein, the behavior of the alamethicin channel was unaffected by increasing the length of fatty acid chains (Keller et al., 1993).

In summary, we report here that altering the physicochemical nature of the environment in which the BK channel is embedded alters its behavior. Although insufficient by itself, structural stress produced in bilayers may be an important mechanism that contributes significantly to changes in ion channel function when the lipid composition around it changes.

This research was funded in part by a grant (82-2687) from the Arizona Disease Control Research Commission, and a BRSG grant (to R.G.). R.R. was supported by National Institutes of Health Training grant HL-07249.

References

Chang, H.-M., Reitstetter, R., Mason, R.P., Gruener, R. 1995. Attenuation of channel kinetics and conductance by cholesterol: an interpretation using structural stress as a unifying concept. J. Membrane Biol. **143:**51–63

Chong, P.L.-G., Fortes, P.A.G., Jameson, D.M. 1985. Mechanisms of inhibition of (Na,K)-ATPase by hydrostatic pressure studied with fluorescent probes. *J. Biol. Chem.* **260:**14484–11490

Colquhoun, D., Sigworth, F.J. 1983. Chapter 11. *In:* Single-Channel Recording. B. Sakmann and E. Neher, editors. pp. 191–263. Plenum, New York

Evans, E., Needham, D. 1987. Physical properties of surfactant bilayer membranes: thermal transition, elasticity, rigidity, cohesion and colloidal interactions. *J. Phys. Chem.* **91:**4219–4228

Fong, T.M., McNamee, M.G. 1986. Correlation between acetylcholine receptor function and structural properties of membranes. *Biochemistry* **25:**830–840

Gibson, N.J., Brown, M.F. 1993. Lipid headgroup and acyl chain composition modulate the MI–MII equilibrium of rhodopsin in recombinant membranes. *Biochemistry* **32:**2438–2454

Gruner, S.M. 1985. Curvature hypothesis: does the intrinsic curvature determine biomembrane lipid composition. A role for non-bilayer lipids. *Proc. Natl. Acad. Sci. USA* **82:**3665–3669

Gruner, S.M. 1992. Nonlamellar lipid phases. Chap. 5. *In:* The structure of biological membranes. P. Yeagle, editor. pp. 211–250. CRC, Boca Raton, Florida

Gruner, S.M., Tate, M.W., Kirk, G.L., So, P.T.C., Turner, D.C., Keane, D.T., Tilcock, C.P.S., Cullis, P.R. 1988. X-ray diffraction study of the polymorphic behavior of N-methylated dioleoylphosphatidylethanolamine. *Biochemistry* **27:**2853–2866

Helfrich, W. 1973. Elastic properties of lipid bilayers: theory and possible experiments. Z. *Naturforsch.* **28c:**693–703

Israelachvili, J. 1991. Chap. 14. *In:* Intermolecular and surface forces, 2nd ed., pp 288–311. Academic, New York

Keller, S., Bezrukov, M., Gruner, S.M., Tate, M.W., Vodyanoy, I., Parsegian, V.A. 1993. Probability of alamethicin conductance states varies with nonlamellar tendency of bilayer phospholipids. *Biophys. J.* **65:**23–27

Krueger, B.K., Worley, J.F., French, R.J. 1983. Single sodium channel from rat brain incorporated into planar lipid bilayers. *Nature* **303:**172–175

Labarca, P., Coronado, R., Miller, C. 1980. Thermodynamic and kinetic studies of the gating behavior of a potassium-selective channel from the sarcoplasmic reticulum membrane. *J. Gen. Physiol.* **76:**397–424

Lewis, R.N.A.H., Mannock, D.A., McElhaney, R.N., Turner, D.C., Gruner, S.M. 1989. Effect of fatty acyl chain length and structure on the lamellar gel to liquid-crystalline and lamellar to reversed hexagonal phase transitions of aqueous phosphatidylethanolamine dispersions. *Biochemistry* **28:**541

Marsh, D. 1990a. Section II. 14 *In:* Handbook of lipid bilayers pp. 265–273. CRC, Boca Raton, Florida

Marsh, D. 1990b. Section II. 7 *In:* Handbook of lipid bilayers p. 151. CRC, Boca Raton, Florida

Marsh, D. 1990c. Section II. 8 *In:* Handbook of lipid bilayers pp. 163–183. CRC, Boca Raton, Florida

McElhaney, R.N. 1989. The influence of membrane lipid composition on physical properties of membrane structure and function in *Achole-plasma laidlail. CRC Crit. Rev. Microbiol.* **17:**1–32

Methfessel, C., Boheim, C. 1982. The gating of single calcium-dependent potassium channels is described by an activation-blockage mechanism. *Biophys. Struct. Mech.* **9:**35–60

Moczydlowski, E., Latorre, R. 1983. Gating kinetics of Ca^{2+}-activated K^+ channels from rat muscle incorporated into planar lipid bailers: evidence for two voltage-dependent Ca^{2+}-binding reactions. *J. Gen. Physiol.* **82:**511–542

Needham, D., Evans, E. 1988. Structure and mechanical properties of giant lipid (DMPC) vesicle bilayers from 20°C below to 10°C above the liquid crystal-crystalline phase transition at 24°C. *Biochemistry* **27:**8261–8269

Needham, D., Nunn, R.S. 1990. Elastic deformation and failure of lipid bilayer membranes containing cholesterol. *Biophys. J.* **58:**997–1009

Rand, R.P., Parsegian, V.A. 1992. Chap. 6, *In:* The structure of biological membranes P. Yeagle, editor. pp. 251–306. CRC, Boca Raton, Florida

Reinhart, P.H., Chung, S., Martin, B.L., Brautigan, D.L., Levitan, I.B. 1991. Modulation of calcium-activated potassium channels from rat brain by protein kinase A and phosphatase 2A. *J. Neurosci.* **11:**1627–1635

Seddon, J.M. 1990. Structure of the inverted hexagonal (H_{II}) phase, and non-lamellar phase transition of lipids. *Biochim. Biophys. Acta* **1031:**1–69

Starling, A.P., East, J.M., Lee, A.G. 1993. Effects of phosphatidylcholine fatty acyl chain length on calcium binding and other functions of the (Ca^{2+} – Mg^{2+})-ATPase. *Biochemistry* **32:**1593–1600

Tate, M.W., Shyamsunder, E., Gruner, S.M., D'Amico, K.L. 1992. Kinetics of the lamellar-inverse hexagonal phase transition determined by time-resolved x-ray diffraction. *Biochemistry* **31:**1081–1092

Toro, L., Vaca, L., Stefani, E. 1991. Calcium-activated potassium channels from coronary smooth muscle reconstituted in lipid bilayers. *Am. J. Physiol.* **260:**H1779–1789

Trave, G., Quignard, J.F., Lionne, C., Sri Widada, J., Liautard, J.P. 1994. Interdependence of phospholipid specificity and calcium binding in annexin I as shown by site-directed mutagenesis. *Biochim. Biophys. Acta* **1025:**215–222

Whitesell, R.R., Regen, D.M., Beth, A.H., Pelletier, D.K., Abumrad, N.A. 1989. Activation energy of the slowest step in the glucose carrier cycle: break at 23°C and correlation with membrane lipid fluidity. *Biochemistry* **28:**5618–5625