The Reduction in Electroporation Voltages by the Addition of a Surfactant to Planar Lipid Bilayers

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ABSTRACT The effects of a nonionic surfactant, octaethyleneglycol mono *n*-dodecyl ether ($C_{12}E_8$), on the electroporation of planar bilayer lipid membranes made of the synthetic lipid 1-pamitoyl 2-oleoyl phosphatidylcholine (POPC), was studied. High-amplitude (~100–450 mV) rectangular voltage pulses were used to electroporate the bilayers, followed by a prolonged, low-amplitude (~65 mV) voltage clamp to monitor the ensuing changes in transmembrane conductance. The electroporation thresholds of the membranes were found for rectangular voltage pulses of given durations. The strength-duration relationship was determined over a range from 10 μs to 10 s. The addition of $C_{12}E_8$ at concentrations of 0.1, 1, and 10 μM to the bath surrounding the membranes decreased the electroporation threshold monotonically with concentration for all durations ($p < 0.0001$). The decrease from control values ranged from 10% to 40%, depending on surfactant concentration and pulse duration. For a 10-μs pulse, the transmembrane conductance 150 μs after electroporation (G_{150}) increased monotonically with the surfactant concentration ($p = 0.007$ for 10 μM $C_{12}E_8$). These findings suggest that $C_{12}E_8$ incorporates into POPC bilayers, allowing electroporation at lower intensities and/or shorter durations, and demonstrate that surfactants can be used to manipulate the electroporation threshold of lipid bilayers.

INTRODUCTION

A lipid membrane, whether cellular or artificial, can be made highly permeable and undergo a loss of integrity when exposed to a high-intensity transmembrane potential (Neumann et al., 1989). The high potential can cause biomembranes to enter a state of reversible electrical breakdown (REB) that is highly permeable to ions and macromolecules (Neumann and Rosenheck, 1972), or cause artificial planar bilayer lipid membranes (BLMs) to undergo either REB or rupture (Tsong, 1991). This phenomenon is known as electroporation.

The physicochemical basis of electroporation is yet to be resolved, but there have been numerous theoretical studies. Most investigations invoke one of two mechanisms to describe electroporation. The first mechanism is based on an electromechanical instability, first proposed by Crowley (1973), stating that the membrane electroporates at a critical field strength dependent on membrane physical properties. As reviewed by Zimmermann (1982), those theories that assume an isotropic membrane predict a critical field strength that is of the correct magnitude, but a thickness change owing to electrocompression that is unrealistically large (Crowley, 1973). These discrepancies can be resolved by assuming the membrane to be anisotropic in its elastic response to deformation and shear (Maldarelli and Stebe, 1992). Another mechanism, based on nucleation theory, considers electroporation as a stochastic process in which a transmembrane potential acts to reduce the energy barrier for the formation of critical pores in the membrane (Weaver and Chizmadzhev, 1996; Abidor et al., 1979; Weaver, 1995). This approach relates the probability of critical pore formation to membrane physical properties such as surface tension, line tension, and transmembrane voltage via an assumed formulation for the free energy of the bilayer. In the current study, the transmembrane potential required to rupture a bilayer for a given waveform and membrane composition is referred to as the electroporation threshold.

Electroporation has been utilized in biotechnology. It is commonly used for gene transfection (Wolf et al., 1994), membrane protein insertion (Mouneimne et al., 1989), and injection of normally membrane-impermeant molecules into cells (Sixou and Teissie, 1992; Neumann and Rosenheck, 1972). Electrofusion of cells is most efficient after the electroporation of the cell membranes (Hui et al., 1996; Neumann et al., 1989). Electroporation also enhances the effectiveness of drugs, especially anticancer drugs (Miklavcic et al., 1997; Mir, 1994), and has promise in transdermal drug delivery (Prausnitz et al., 1993).

With such applications in mind, there is clearly interest in applying electroporation to living tissue. However, the high-intensity fields needed for electroporation can have undesired side effects. In vivo, there is often irreversible damage to the exposed cells and tissue by Joule heating (Lee and Kolodney, 1987). Increasing electric fields in vitro diminishes cell survival rates (Gabriel and Teissie, 1995; Wolf et al., 1994). A means of increasing a cell membrane's susceptibility to electroporation may be a way of alleviating in vivo thermal injury and increasing in vitro cell survival rates. The most efficient electroporation conditions involve a proper choice of intensity and duration of electrical stimulus (Hui, 1995). Another element that could influence the

Received for publication 12 January 1998 and in final form 12 May 1998.

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0006-3495/98/08/880/09 $2.00

efficacy of electroporation is a chemical modification of the cell membranes. For example, the addition of the triblock copolymer poloxamer 188 to the membrane raises the electroporation threshold of a given waveform (Sharma et al., 1996) and promotes postelectroporation wound healing (Lee et al., 1992). Adding smaller surfactants that adsorb into the membrane to lower the electroporation threshold may be another such manipulation, and was the goal of this study.

Surfactants are widely used as agents to interact with membranes. Being amphiphilic, surfactants tend to adsorb to membranes and interfaces, reduce surface tensions, and above the critical micellar concentration (CMC) form micelles. Polyoxyethylene alcohols (C_nE_m) are a synthetic group of nonionic surfactants. They contain an aliphatic hydrocarbon tail, C_n, and a hydrophilic headgroup of *m* oxyethylene units, $(OCH_2CH_2)_m$-OH. The polyoxyethylene alcohol octaethyleneglycol *n*-monododecyl ether ($C_{12}E_8$) was used in this study in concentrations under its CMC, which is 71 μM (Garrett and Grisham, 1995; Lin et al., 1997). This surfactant incorporates into lipid bilayers (Heerklotz et al., 1996). The BLMs used in this study were composed of a single lipid, 1-pamitoyl 2-oleoyl phosphatidylcholine (POPC), to avoid difficulties inherent with mixtures of lipids in regard to experimental reproducibility. POPC is a zwitterionic lipid and a common constituent in biological membranes (Garrett and Grisham, 1995), and, in isolation, prefers to exist in the bilayer state (Israelachvili, 1985).

The electroporation threshold at which a lipid membrane ruptures has been shown to depend on the bilayer composition (Needham and Hochmuth, 1989; Chernomordik et al., 1987; Sharma et al., 1996), the duration of the stimulus (Abidor et al., 1979), and other stresses in the bilayer (Needham and Hochmuth, 1989). During BLM rupture, the transmembrane conductance increases until it reaches that of a membrane-free state. The process often has two or more phases with different characteristic time constants (Kinosita and Tsong, 1977; Sharma et al., 1996). Changing membrane composition and stimulus intensity can also change the time scales and relative contributions of these two phases (Sharma et al., 1996).

In this study, the effect of a monodisperse surfactant, $C_{12}E_8$, on the electroporation of a pure POPC lipid bilayer was investigated. The electroporation threshold was found for each duration of the rectangular pulses used, and changes in these thresholds were recorded for each surfactant concentration. The characteristic time scales for membrane rupture were also found as a function of surfactant concentration and stimulus amplitude.

MATERIALS AND METHODS

The lipid 1-pamitoyl 2-oleoyl phosphatidylcholine (POPC) was obtained (Avanti Polar Lipids, Alabaster, AL) either in a solution of 10 mg/ml in chloroform, or in powder form, from which a solution of 10 mg/ml in a 9:1 mixture of hexane and ethanol was made. Octaethyleneglycol mono *n*-dodecyl ether ($C_{12}E_8$) was obtained in crystal form (Barnet Products Corporation, Englewood Cliffs, NJ) via Nikko Chemicals (Tokyo, Japan). Deionized water, filtered to remove organic impurities, with a resistivity of 18 MΩ·cm, was used to make all solutions and in all cleaning procedures.

Planar bilayers were formed by the folding method (Ehrlich, 1992) across a circular hole 105 μm in diameter. The hole was formed in a 25 μm thick Teflon sheet by a high-voltage electrical discharge across the sheet. The experimental setup was similar to that used previously (Sharma et al., 1996), except that the lipid bilayer chamber was made of Teflon, and several improvements were made in its design (Fig. 1). The advantage of Teflon as the chamber material was its high resistance to chemicals, allowing the use of strong solvents and cleaning agents. Other improvements of the chamber included the tapering of the walls leading to the hole, resulting in a lower dependence of stray capacitance on the level of the solution; electrode insertion from the side, allowing the electrodes to remain below the solution level; and insertion of a glass window for easy viewing of the hole.

The chamber was soaked in sulfuric acid between experiments, rinsed with running water, and allowed to dry before each use. Before being mounted in the chamber, the Teflon sheet was washed under running water, soaked in ethanol, washed with water again, and submerged in chloroform for at least 20 min after drying. The four electrodes for the stimuli and measurements of current and voltage were placed in agar bridges and

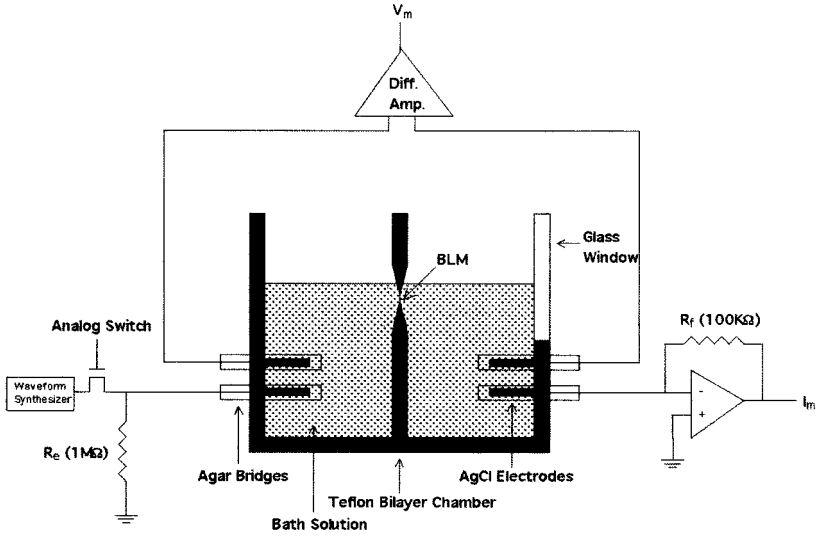

FIGURE 1 Schematic of the experimental setup. The Teflon sheet was mounted in the Teflon bilayer chamber such that the hole on which BLMs were formed was exposed to the bath solution. The setup employed four Ag-AgCl electrodes, inserted via agar bridges below the solution level. Two of these electrodes were used to measure transmembrane voltage (V_m) via a high-impedance differential amplifier, and the remaining two electrodes were used to apply voltage pulses across the membrane and measure transmembrane current (I_m). The analog switch isolated the membrane from the voltage source after the application of a pulse and allowed membrane voltage to decay through an external 1-MΩ resistor (R_e). The glass window allowed easy viewing of the hole during membrane formation.

mounted in the chamber. The agar bridges were made from glass pipettes and filled with a gel consisting of 2% agarose (Sigma Chemical Company, St. Louis, MO) in 3 M KCl.

Both sides of the hole in the Teflon sheet were treated first with 1 μl of the 10 mg/ml lipid solution, which made it possible to form more stable bilayers. After the lipid solvent completely evaporated, the hole was treated with 1–2 μl of hexadecane (30% in pentane) on each side. Each compartment was filled to just below the hole (~1.5 ml) with salt solution (100 mM KCl, 10 mM HEPES, pH 7.40). A drop of 2 μl of the lipid solution was carefully deposited on each side of the chamber and allowed to spread over the aqueous-air interface for at least 15 min. To form a bilayer, the solution level in both compartments was carefully raised to the same height just above the hole, to eliminate any hydrostatic pressures exerted on the membrane caused by differences in solution levels. After a bilayer was ruptured by irreversible electroporation, a new one was formed by lowering the solution levels below the hole, and then raising them again. All experiments were performed at room temperature (22–24°C).

The membrane capacitance (C_m) and stray capacitance of the circuitry, chamber, and sheet were measured from charge pulse traces as previously described (Sharma et al., 1996). The system was pulsed for 10 μs at an amplitude below the electroporation threshold, usually 300 mV or less. Specific capacitance (C_{sp}) was estimated for the first three or four membranes of each experiment by dividing C_m by the membrane surface area, 7.79×10^{-5} cm^2. The membrane was assumed to span 90% of the area of the hole (8.66×10^{-5} cm^2), with the remaining 10% of the hole area occupied by the torus of solvent that supports the membrane.

A voltage pulse was used to electroporate the membranes. This pulse lasted for one of seven durations that varied by factors of 10 over the range of 10 μs to 10 s. The amplitude was incremented in steps of 5–10 mV from an initial subthreshold intensity, ~80 mV below the expected threshold for the solution conditions and pulse duration used, until electroporation took place. The expected threshold was determined either from data from previous experiments or by stimulating a membrane with pulses in coarser increments of ~25 mV. This made it possible to subject membranes to approximately the same number of pulses before electroporation occurred. The voltage that caused electroporation was recorded as the electroporation threshold of that membrane. Immediately after the pulse the membrane was clamped to ~65 mV for the duration of the pulse or for 500 μs, whichever was greater. The purpose of the clamp was to record the conductance rise of the ruptured membranes, calculated as the transmembrane current (I_m) divided by the transmembrane voltage (V_m). The time between the beginning of the pulse and the onset of the conductance rise was defined as the latency time. The conductance rise after the 10-μs pulses was also characterized by the parameter G_{150}, which was the transmembrane conductance 150 μs after the onset of the pulse. Because the resistance of the membrane decreased dramatically upon rupture, the transmembrane conductance had to be corrected for the hole conductance (see Appendix). The conductance of the bare hole was recorded by applying the low-intensity voltage clamp after membrane rupture.

All membranes were checked for stability with subthreshold pulses for at least 5 min before experimentation. Before each experiment, at least five bilayers were formed and monitored for threshold reproducibility and stability over a time of 2 h or more. If these conditions were satisfied, at least five bilayers were electroporated at each pulse duration.

For surfactant experiments, after membrane stability and threshold reproducibility were ensured, 15 μl of $C_{12}E_8$ solution at 100 times the desired concentration (0.001–100 μM) was injected into one of the compartments of the chamber, which contained 1.5 ml of solution at a height just below the hole. Bilayers were formed as before, and after steady state was reached, electroporation measurements were made as described for control conditions. Some experiments were also performed by adding surfactant to the bath solution at least 1 h before lipid deposition. The surface was aspirated before lipid deposition in these experiments, so that there was very little surfactant at the interface while the lipid spread.

The statistical significance of changes in parameters after changing durations and/or surfactant concentrations was determined using pooled Student's *t*-tests. Groups of data were also compared with analysis of covariate (ANCOVA) analysis, using duration as a continuous variable and concentration as a discrete factor. The p values of less than 0.05 were considered significant for rejection of the null hypothesis.

RESULTS

The first parameter compared for control and $C_{12}E_8$-treated membranes was capacitance. The specific capacitance (C_{sp}) of control membranes was measured to be 0.59 ± 0.15 μF/cm^2 (n = 25). The C_{sp} values for membranes treated with $C_{12}E_8$ were 0.60 ± 0.12 (n = 10), 0.57 ± 0.16 (n = 10), and 0.60 ± 0.11 μF/cm^2 (n = 10) for 0.1, 1, and 10 μM $C_{12}E_8$, respectively, and were not statistically different from control values.

A typical current trace of a subthreshold voltage pulse and clamp (Fig. 2 *A*) had only capacitive spikes at the two voltage step changes. The intact membrane was highly impermeable to ions and therefore had negligible conductance. In contrast, when a pulse was of high enough amplitude and long enough duration to elicit electroporation, the current trace was seen to rise during or immediately after the pulse (Fig. 2 *B*). This current rise indicated the increasing conductance as the membrane ruptured.

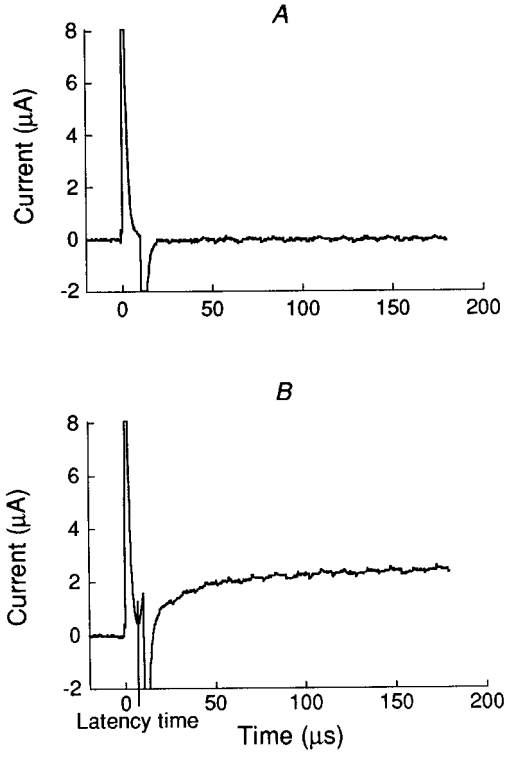

FIGURE 2 The transmembrane current trace of a voltage pulse (10 μs) followed by a voltage clamp of 61 mV (180 μs). (*A*) The pulse intensity was 400 mV, which does not elicit membrane rupture. There is no current except for the two capacitive spikes at the onset of the pulse and onset of the clamp. (*B*) The pulse intensity was 449 mV, which elicits electroporation and membrane rupture. The two capacitive spikes are still present, along with a current rise at the onset of electroporation. The time from the beginning of the pulse (<0.5 μs) to the initiation of the current rise was interpreted as the latency time, which in this case was 7.2 μs. Both traces have been truncated in amplitude.

TABLE 1 Electroporation thresholds for each $C_{12}E_8$ concentration and pulse duration

Duration	Control	0.1 μM	1 μM	10 μM
10 μs	450 ± 24	383 ± 31	333 ± 22	301 ± 24
100 μs	398 ± 19	346 ± 12	273 ± 22	248 ± 33
1 ms	331 ± 20	264 ± 25	235 ± 21	227 ± 21
10 ms	282 ± 26	238 ± 22	205 ± 16	192 ± 22
100 ms	258 ± 9	204 ± 18	165 ± 22	154 ± 27
1 s	213 ± 18	158 ± 21	162 ± 18	122 ± 10
10 s	167 ± 6	151 ± 19	136 ± 11	112 ± 7

All pairs of thresholds grouped with a bracket (]) were compared using a Student's *t*-test, and those labeled by an asterisk (*) are statistically different ($p < 0.05$). All thresholds for surfactant are significantly different from control thresholds at the same duration ($p = 0.0007$ at 10 ms and 0.1 μM $C_{12}E_8$, $p = 0.02$ at 10 s and 0.1 μM $C_{12}E_8$, $p < 0.0001$ at all other durations and concentrations). For all data points, $n \geq 10$.
Values are in mV, mean ± SD.

After surfactant injection, bilayers were electroporated approximately every 10 min until a reproducible threshold was reached, typically 60–100 min after the injection. When surfactant was already present in the bath, a reproducible threshold of electroporation was reached some 60–100 min after lipid deposition and was the same as that realized for the injection protocol. This agreement suggests that the transport of $C_{12}E_8$ is not limiting in the injection protocol and that some time is required for the surfactant to fully adsorb into the lipid complex. Stable bilayers could not be formed for surfactant concentrations of 100 μM or greater. For the membranes treated with a surfactant solution of 0.01 μM or less, the strength-duration relationship remained unchanged from that of control.

Table 1 is a comparison of average and standard deviations of threshold voltages for each pulse duration and surfactant concentration. The threshold voltage decreased with increasing pulse duration. For pulse durations up to 1 s, a 10-fold increase in pulse duration at fixed $C_{12}E_8$ concentration resulted in a statistically significant decrease in threshold voltage ($p < 0.05$), except for one data point (1 s pulse at 1 μM $C_{12}E_8$). The strength-duration data are also presented in graphical form (Fig. 3 *A*). Here error bars in the electroporation threshold represent the standard deviations. Duration error bars stretch back in time from the duration of the pulse to the average latency time for each group of membranes. Latency times were typically half of the pulse duration.

For control data, the decrease in electroporation thresholds with increasing duration was linear on the semilog plot. Over the concentration range of 0.1 to 10.0 μM, increasing levels of $C_{12}E_8$ decreased thresholds for a given pulse duration. This resulted in a shift in the strength-duration curve downward and to the left (Fig. 3 *A*). Thresholds for 0.1 μM $C_{12}E_8$ were significantly different from those of control at all pulse durations ($p = 0.0007$ at 10 ms, $p = 0.02$ at 10 s, $p < 0.0001$ at all other durations). The strength-duration relationship for all three surfactant concentrations was significantly different from that of control and from each other by ANCOVA analysis ($p < 0.0001$). Fig. 3 *B* summarizes the effect of $C_{12}E_8$ on the electroporation threshold for all concentrations tested. The curve represents a Langmuir isotherm, where the effect (% decrease) is equal

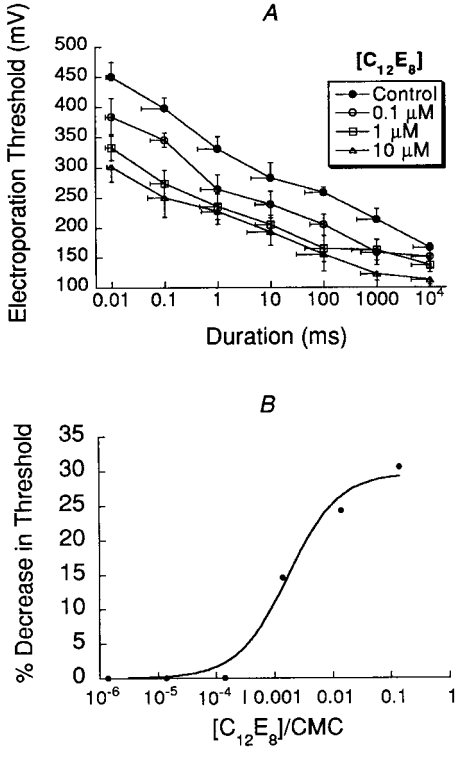

FIGURE 3 (*A*) Electroporation thresholds versus pulse duration for each $C_{12}E_8$ concentration. Data from at least 10 membranes were averaged for each point. Error bars in threshold represent standard deviations of the data. Error bars in duration reach back from pulse duration to average membrane latency time. All four sets of data collectively were significantly different from each other by ANCOVA analysis ($p < 0.0001$). (*B*) The percentage decrease in electroporation threshold plotted as a function of $C_{12}E_8$ concentration, normalized by its CMC (71 μM). The percentage decrease in electroporation thresholds was calculated by fitting the strength-duration data to a semilogarithmic curve with a slope of 45 mV/decade in time, and calculating the percentage shift of the intercept from control. The curve is the best fit for a Langmuir isotherm, with the form (% decrease) = $K_1 \times [C_{12}E_8]/([C_{12}E_8] + K_2)$.

to $K_1 \times [C_{12}E_8]/([C_{12}E_8] + K_2)$. K_1 and K_2 were estimated to be 30 and 1.7 μM, respectively.

Transmembrane conductance was computed as I_m/V_m. Fig. 4 shows a typical conductance rise after a 10-μs pulse on short and long time scales, compared with the reference conductance of the bare hole. The post-electroporation conductance of control membranes 150 μs after a 10-μs pulse, G_{150}, was 58 $\pm$ 13 μS (Fig. 4 *A*). The conductance rise could not be described by a single exponential for the entire time course (Fig. 4 *B*). The addition of $C_{12}E_8$ accelerated the conductance rise, resulting in higher values for G_{150}, as summarized in Table 2. The increase in G_{150} was statistically significant ($p = 0.007$) at a concentration of 10 μM $C_{12}E_8$, and represented a change of 50%.

To test the hypothesis that the increase in G_{150} was caused by $C_{12}E_8$ and not by a decrease in pulse amplitude owing to lower thresholds, the effect of pulse amplitude on the conductance rise for control membranes was also investigated for 10-μs pulses. The effect of a decrease in pulse amplitude could not be studied, because the pulses used were just above the threshold; so the effect of an increase in amplitude was tested. A statistically significant increase was found in values of G_{150} for control membranes when the intensities of 10-μs electroporating pulses were increased (Table 2), suggesting that lower values of G_{150} occur at lower pulse amplitudes. Because $C_{12}E_8$-treated bilayers were subjected to lower amplitude electroporating pulses, it is presumed that increases in G_{150} for surfactant-treated bilayers were caused by the incorporation of $C_{12}E_8$ and not by the decrease in pulse amplitude. G_{150} values for 10 μM $C_{12}E_8$-treated bilayers electroporated at their 10-μs threshold (~300 mV) were similar to G_{150} values for control membranes subjected to pulses of 500 mV (11% above threshold).

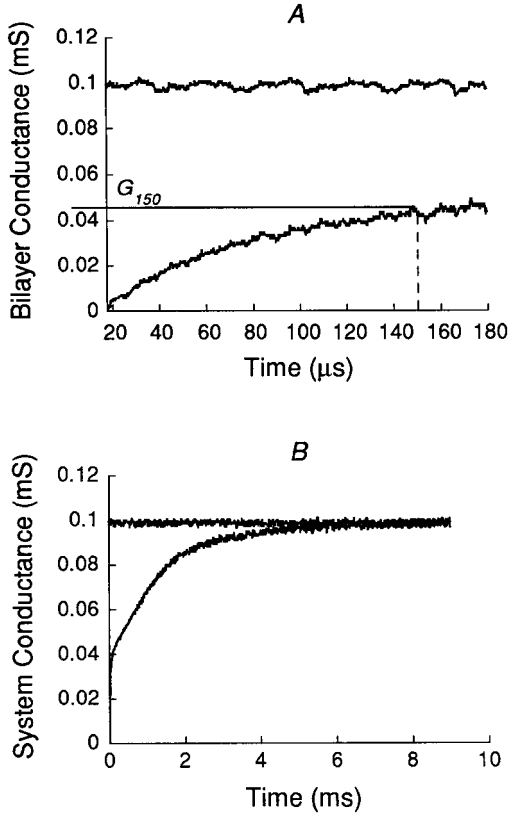

FIGURE 4 The conductance rise of a membrane ruptured by a 10-μs electroporating pulse, which was onset at $t = 0$. The conductance of the bare hole (~0.1 mS) is also plotted. (*A*) The conductance rise onset and first phase can be seen on a relatively short time scale. The conductance reached at 150 μs is labeled G_{150}. (*B*) Additional phases of conductance rise can be seen, and the approach of the system conductance to that of the bare hole can be seen on a longer time scale.

TABLE 2 G_{150} for each $C_{12}E_8$ concentration and for POPC bilayers with different pulse amplitudes

Concentration (μM)	G_{150} (μS)
0.0 (control)	58 $\pm$ 13
0.1	64 $\pm$ 16
1.0	68 $\pm$ 20
10.0	87 $\pm$ 29 ($p = 0.007$)
Control at 500 mV	80 $\pm$ 32 ($p = 0.045$)
Control at 600 mV	170 $\pm$ 46 ($p = 0.0001$)
Control at 800 mV	460 $\pm$ 110 ($p = 0.0001$)

For control and surfactant experiments, G_{150} was measured after a 10-μs pulse just above threshold (pulses were incremented by 5–10 mV). For all data points, $n \geq 10$.
Values are mean $\pm$ SD.

DISCUSSION

This study includes a manipulation of bilayer composition by using a synthetic surface-active agent with the purpose of increasing the membrane susceptibility to electroporation. It also includes a complete strength-duration analysis that covers seven orders of magnitude in duration on BLMs composed of a single lipid. The surfactant $C_{12}E_8$ was found to significantly alter the electroporation parameters for POPC lipid bilayers over a concentration range of 0.1–10 μM. For 10-μs pulses, pure POPC bilayers ruptured at a mean voltage of 450 mV. This threshold dropped to 383 mV (a 15% decrease), 333 mV (26%), and 301 mV (33%) upon the addition of 0.1, 1, and 10 μM $C_{12}E_8$, respectively. These decreases remained relatively constant over the range of pulse durations tested. For 10-s pulses, electroporation thresholds dropped from 167 mV to 151 mV (a 10% decrease), 136 mV (19%), and 112 mV (33%), respectively. The membrane conductance shortly after a 10-μs pulse with amplitude just above threshold increased with increasing $C_{12}E_8$ concentration, as reflected by changes in G_{150}. There was also an increase in G_{150} observed for control membranes for increasingly suprathreshold pulse amplitudes.

Strength-duration relationship

A dependence of membrane latency time on stimulus amplitude was suggested shortly after the discovery of electroporation (Tien, 1974). Typically, an amplitude is selected,

and the bilayer is held at that value until membrane breakdown occurs (Chernomordik et al., 1985; Abidor et al., 1979). A wide range of relationships between latency time and amplitude has been found, but all are consistent in reporting a decline in latency time with an increase in stimulus amplitude. Most of the data have at least some portion that is linear on a semilogarithmic plot. Depending on the lipids and solvents used, the slopes of these relationships have ranged from 400 mV/decade of time (Chernomordik, 1992) to 25 mV/decade (Chernomordik et al., 1985), with typical values between 30–80 mV/decade (Chernomordik et al., 1985; Abidor et al., 1979; Chernomordik, 1992; Chizmadzhev and Pastushenko, 1988).

This study used a slightly different approach to obtain the electroporation strength-duration relationship. It was based on finding electroporation thresholds and latency times for rectangular pulses of different stimulus durations, somewhat like the procedure used for charge pulse experiments (Wilhelm et al., 1993). Unlike in the charge pulse experiments, however, membrane breakdown in our study always took place during and not after the pulse stimulus. Furthermore, the voltage was clamped during the pulse, which could be chosen to be any duration, unlike in the charge pulse method, which is restricted to very short duration pulses. One advantage of this protocol is an improved resolution of the strength-duration relationship, particularly for relatively long pulses, for which thresholds do not change much. Moreover, in practical applications of electroporation, the duration of the stimulus must be specified before it is employed, so it would be useful to know the threshold of the membrane for the pulse width chosen. Another advantage in finding the threshold as a function of duration is that the experimentalist knows the time frame within which the bilayer will electroporate. The time base of the data recording system can be chosen accordingly to enable the dynamics of the electroporation process to be monitored at the highest possible time resolution.

In this study, electroporation thresholds for most of the durations and surfactant concentrations were found to decrease linearly with the logarithm of time with a slope of ~45 mV/decade. For control experiments with pure POPC bilayers, this semilogarithmic relation held for even the longest of clamping durations possible with the apparatus, 10 s. After the addition of $C_{12}E_8$, the slope decreased to as low as 10 mV/decade in the duration range of 1–10 s.

Strength-duration models

Both the electromechanical and nucleation theories predict the trend of decreasing electroporation thresholds with pulse duration. However, only the functional form for the nucleation theory available for predicting threshold versus duration (Pastushenko et al., 1979) agrees well with our data.

Dimitrov's (1984) stability analysis predicts a dependence on electric pulse length to the electroporation thresholds of the electromechanical theory. It predicts a minimum critical voltage, U_c, for which the membrane becomes unstable at long pulse durations ($U_c = (8\sigma E h^3/\epsilon_m^2\epsilon_o^2)^{1/4}$, where σ is the membrane surface tension, E is the elastic modulus of the membrane, h is the membrane thickness, ϵ_m is the dielectric constant of the membrane, and ϵ_o is the permittivity of free space). Assuming that $E = 10^7$ Nm^{-2} (consistent with the data of Needham and Hochmuth for SOPC; Needham and Hochmuth, 1989), $\sigma = 10^{-4}$ Nm^{-1}, $h = 5 \times 10^{-9}$ m, $\epsilon_m = 2.24$, and $\epsilon_o = 8.85 \times 10^{-12}$ Fm^{-1}, U_c is estimated to be 1.26 V, an order of magnitude higher than the values observed in our experiments. Even when U_c is chosen to be on the order of the electroporation thresholds found at the longest clamping duration (10 s), the shape of the predicted curve does not match well with the data (R values Å 0.8). However, decreases in electroporation thresholds owing to the presence of surfactant do agree with this theory qualitatively, if it is assumed that surfactants decrease E and σ, and do not change the other parameters. The decrease in E may be expected because single-chain surfactants generally facilitate the area expansivity of membranes (Zhelev, 1996). Decreases in membrane surface tension would be expected based on that which is found in monolayer experiments (Sundaram and Stebe, 1997). Unchanged C_{sp} values indicate that h and ϵ_m are unchanged after the incorporation of $C_{12}E_8$.

For the nucleation theory, if the capacitance is constrained to our experimentally observed values, the strength-duration data for our control and $C_{12}E_8$-treated membranes can be fit by the membrane-lifetime equation with the steady-state diffusion approximation (Pastushenko et al., 1979). There are three unknown parameters: σ, the membrane surface tension; γ, the pore line tension; and D, the pore radii diffusion constant (or c_oAD, where A is the unit area of the membrane, for membranes with c_o defects). If all three parameters are fit by a best-fit algorithm for the means of the strength-duration data, the trends observed are decreases in σ and γ with increasing $C_{12}E_8$ concentration. These trends become more pronounced when c_oAD is constrained to be constant for all four sets of data (Fig. 5). For these fits σ is between 3.0 and 8.4×10^{-4} Nm^{-1}, and γ between 1.1 and 1.8×10^{-11} N, which is on the order of their accepted values (Chernomordik et al., 1985). It should be noted, however, that very small changes in these parameters cause very large changes in the relationships (see figure 1 of Pastushenko et al., 1979), and the predictions can also fail to fit the data with accepted parameter values.

Membrane conductance

It is apparent from Fig. 4 *B* that the conductance rise of a system with a rupturing BLM takes place in at least two phases. A biexponential fit ($G = A \times (1 - e^{-t/\tau_1}) + B \times (1 - e^{-t/\tau_2})$) had an R value of 0.996, better than that of a single exponential ($G = A \times (1 - e^{-t/\tau_1})$; $R = 0.937$). A rapid phase on the order of 100 μs ($\tau_1 = 0.033$ ms) was followed by a slower phase ($\tau_2 = 1.32$ ms) in which the

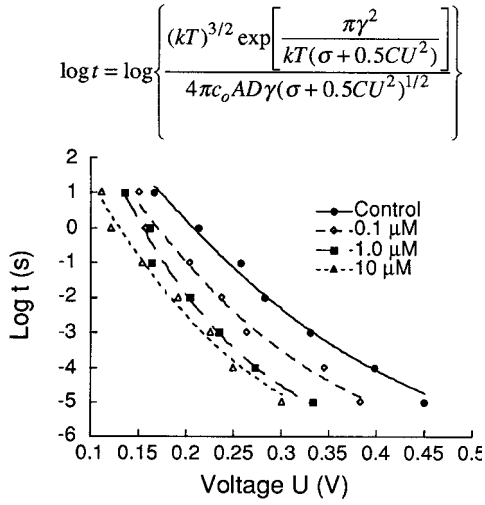

FIGURE 5 Curve fits of our data to the stochastic theory (Pastushenko et al., 1979), and the equation used. The log t is plotted against the voltage V. For all sets of data, c_o4D was assumed to be 10^{-8} cm^2/s. Here k is Boltzmann's constant, T is temperature, and $C = C_{sp}(\epsilon_s/\epsilon_m - 1)$, where ϵ_s is the permittivity of the aqueous solution. Values found for σ and γ were 8.4, 5.7, 3.0, and 3.6×10^{-4} Nm^{-1} and 1.8, 1.4, 1.14, and 1.12×10^{-11} N for control, 0.1, 1, and 10 μM $C_{12}E_8$, respectively.

conductance approached that of the bare hole within 10 ms. This biexponential behavior is similar to that observed by Sharma et al. (1996) for BLM, and to that observed by Kinosita and Tsong (1977) for an erythrocyte suspension, although the time scales are different.

When $C_{12}E_8$ was present in the bilayers, the time scales of the conductance rise after rupture were reduced, as reflected by monotonically increasing G_{150} values with surfactant concentration. The increase in G_{150} is even more significant, considering that $C_{12}E_8$-treated bilayers were electroporated with lower amplitude pulses, whereas G_{150} tends to increase with pulse amplitude (Table 2). Thus, when a $C_{12}E_8$-treated bilayer was electroporated at a voltage near its threshold for some pulse duration, it was conductively similar to a POPC bilayer electroporated with a transmembrane voltage well above its threshold.

$C_{12}E_8$-POPC Interactions

The interaction of $C_{12}E_8$ with POPC bilayers has been shown to depend strongly on bulk concentration (Heerklotz et al., 1996). At the lowest concentrations, below the CMC of 71 μM, some surfactant monomers in solution incorporate into the bilayer. In our study, no change from control in electroporation parameters took place at extremely low concentrations of $C_{12}E_8$ ($\leq$0.01 μM), presumably because the number of incorporated surfactant monomers was not significant enough to change the response of the POPC bilayers to the applied electric field. As the concentration was increased from 0.1 to 10 μM, the decrease in threshold (Fig. 3 *B*) and increase in G_{150} (Table 2) became more pronounced. Assuming that this decrease in threshold is directly proportional to the amount of $C_{12}E_8$ incorporated into the bilayers, the plot of Fig. 3 *B* is consistent with a Langmuir-like adsorption of the surfactant into the bilayer. Finally, Heerklotz et al. (1996) demonstrated that POPC membranes lose their integrity and are completely solubilized for concentrations well above the CMC. In our study, no stable membranes were formed in this concentration range.

Comparison to previous studies

Other studies have reported changes in the electroporation properties of membranes after the addition of amphiphiles, and that the changes depend primarily on the structure of the additive. The addition of the block copolymer poloxamer 188 to the bath surrounding membranes of azolectin increased the thresholds by 5–15% (Sharma et al., 1996). Poloxamer 188 consists of two macromolecular hydrophilic oxyethylene groups, $E_{\sim 75}$, connected by a long hydrophobic propylene oxide chain $(OCHCH_3CH_2)_{\sim 30}$, very different from $C_{12}E_8$ structurally. On the other hand, lyso phosphatidylcholine (LPC), a small, single-chain amphiphile, like $C_{12}E_8$, decreased electroporation thresholds of membranes of egg phosphatidylcholine (Chernomordik et al., 1985). In that study, to achieve the same latency time as with control egg PC bilayers, bilayers treated with 400 μg/ml LPC ($\sim$1 mM) had membrane potentials 100 mV lower on average. This corresponds to a 30–40% decrease in intensity after the addition of LPC, which is similar to the threshold decrease of 30–40% in our study for the highest $C_{12}E_8$ concentration (10 μM). The structural similarities between $C_{12}E_8$ and lysoPC suggest that the increase in susceptibility to electroporation in both cases is related to the conical shape of the single-chain surfactants (Israelachvili, 1985; Chernomordik et al., 1985), which acts to increase the spontaneous curvature of the membrane and decrease the linear tension at the edge of a pore.

Implications

Our study indicates that by judiciously choosing the concentration of surfactant added to bilayers, the time scales for electroporation and the stimulus intensity required can be reduced. Extrapolating this result to biological membranes, a higher degree of membrane permeability may be achieved with a smaller pulse, reducing the thermal injury and other side effects associated with high voltages. In using $C_{12}E_8$ for tissue applications of electroporation, one would also have to keep in mind other possible interactions between $C_{12}E_8$ and membrane proteins. For example, if used in concentrations above the CMC, the surfactant will extract membrane proteins (Levy et al., 1990). It has also been used in concentrations below the CMC to alter the functions of integral proteins, especially ion transport and binding functions (Lu and Kirchberger, 1994; Champeil et al., 1986; Mimura et al., 1993; Highsmith, 1990). Possible mecha-

nisms of such $C_{12}E_8$-protein interactions have been studied (Le Maire et al., 1983; Moller and le Maire, 1993). Provided these side effects are not prohibitive, the use of $C_{12}E_8$ has the promise of conditioning cell membranes before electroporation in biotechnology applications.

This work was supported in part by a Johns Hopkins Whiting School Young Faculty Research Initiative Grant.

APPENDIX

Determination of G_{150}

For an intact bilayer, the high membrane resistance could be expected to dominate the resistance of the hole in the 25-μm Teflon sheet. At 150 μs after electroporation, however, the conductance of the membrane was found to rise to the same order of magnitude as that of the bare hole. Now the system can be modeled as two resistors in series: G_m, the membrane conductance, and G_h, the measured bare hole conductance. We assume that G_h does not change after the formation of a bilayer, because the thickness of the bilayer ($< 10^{-6}$ cm) is much less than the length of the hole (2.5×10^{-3} cm). The total conductance, $G_t = G_m G_h/(G_m + G_h)$, was measured 150 μs after electroporation. G_{150} was calculated from the G_h and G_t as $G_{150} = G_t G_h/(G_h - G_t)$. Note that this measure of conductance differs from that used previously (Sharma et al., 1996), which used a measure of the total conductance (G_t) of the system.

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