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Inhibition of gramicidin channel activity by local anesthetics

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Ondrias et al. ((1986) Stud. Biophys. 115, 17–22) found that dibucaine, butacaine, and tetracaine reduce the conductance of membranes containing multiple ($> 10^6$) gramicidin channels. Similar experiments with local anesthetics (LA's) added to the bath while gently stirring showed that the inhibition developed slowly over a time course of 5–10 min. We developed a many (10–20) channel membrane technique which demonstrated that when LA's were added to the bath and the membrane was repeatedly broken and reformed, the channel occurrence frequency declined promptly. In standard single-channel membrane experiments at lower gramicidin densities, the mean single channel conductance and lifetime distributions with LA's present in the bath did not differ from the controls. The predominant channel conductance amplitude was lower by 9.1% than those of controls, but channel amplitude distributions were also modified so that the net reduction in overall population channel conductance was only about 2.0%. Channel currents showed no evidence of flicker blocks. The lifetime histograms of control and LA-exposed channel populations were both satisfactorily fit by a single-exponential function with the same mean. Thus, inhibition is due primarily to a reduction in the frequency of occurrence of conducting channels, implying a reduced concentration of active monomers in the membrane.

Introduction

Local anesthetics inhibit various membrane currents. For example, Na currents are inhibited by benzocaine, lidocaine, or their derivatives in frog myelinated nerve [28,12,13], squid axon [10,11], rabbit Purkinje fibers [5] and guinea pig cardiac myocytes [8,30]. Delayed rectifier K^+-currents are inhibited by procaine and lidocaine derivatives in squid axon [19,20]. Calcium channel currents in dorsal root ganglion are inhibited by lidocaine and procaine in the snail [2] and frog [24], and tetracaine blocks calcium current in guinea pig ventricular myocytes [8]. Choline flux through calcium-activated cation channels in sarcoplasmic reticulum is blocked by procaine [31].

LA's could inhibit these ion currents at the channel site by occluding the permeation pathway and/or by affecting channel gating [28,13]. Single-channel current measurements are valuable for identifying the most likely mechanism(s). Analysis of single acetylcholine channel currents in frog skeletal muscle exposed to QX drugs (permanently charged forms of lidocaine) reveals that LA's block the channels in the open state [1,26] causing 'flicker' blocks in the current records [21]. LA's cause both fast and slow blocks in BTX-activated sodium channels from rat skeletal muscle [18]. Fast blocks are flicker blocks that are so short that they appear as an increase in single channel noise; slow blocks are manifested as a reduction in the channel occurrence frequency with no affect on the channel lifetime. Lidocaine produces flicker blocks in osmotically-induced potassium single-channel currents from turtle colon [25,9].

The conductance properties of the gramicidin A dimers have made it a valuable model of K^+ and Na^+ channels. The channel is selectively permeable to monovalent cations [17]. Its simple, well defined composition and pore-like geometry [29], capacity to conduct several different monovalent cations, and ease of channel formation in various types of membranes make it a useful and highly manipulable model system for analysis of ion transport and block mechanisms [3].

Recent work has demonstrated that high concentrations of local anesthetic molecules at acidic pH decrease the conductance of gramicidin A-incorporated synthetic bilayers [23]. The explanations suggested by Ondrias et al. for this phenomenon have centered on membrane effects, i.e. intercalation of LA molecules between lipids causing the disordering of lipid tails [22] and the inducement of nonlamellar phases [4]; or, altered membrane surface potentials [18]. Ondrias et

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al. did not measure single channel current properties. By analyzing the single-channel currents, it should be possible to determine whether local anesthetic inhibition is a consequence of a reduction in the conductance amplitude of individual channels; a change in distribution of channel lifetimes; or a reduction in the frequency of occurrence of conducting channels.

Materials and Methods

Solutions and preparation. Gramicidin A solutions used in experiments were made from 1 μg/ml stock solutions of gramicidin A in 86% MeOH, purified from gramicidin D (a natural mixture of gramicidins A, B, and C) by HPLC according to the method outlined by Koeppe and Weiss [15]. LA's were purchased neat, tetracaine and dibucaine in hydrochloric salt form, butacaine in hemisulfate salt form, from Sigma Chemical Company, St. Louis MO. Dibucaine and butacaine solutions were made in 100% MeOH, and tetracaine was dissolved in purified water. Reported LA concentrations are final concentrations of local anesthetic in the measured volumes of bath solutions used in recording chambers. All glassware used in making solutions and the chamber equipment used in experiments was soaked for 5–10 min in $HCrSO_4$ cleaning solution and rinsed immediately afterward with water, acetone, and petroleum ether. Control solutions were equal volumes of solvent without LA dissolved.

Multi-channel membrane conductance measurements. LA concentrations previously reported to yield conductance decrease of approx. 50% in gramicidin A-doped membranes [23] were verified through multi-channel membrane conductance measurements. Synthetic planar lipid bilayer membranes were formed with glyceryl monoolein (NuChek Prep, Elysian, MN) in 50 mg/ml hexadecane, across a partition aperture 830 μm in diameter which joined two reservoirs in a Teflon block. Each reservoir was filled with 1.80 ml unbuffered aqueous 1 M KCl (pH 5.45) and was constantly magnet-stirred with a 10 mm stirring bar. Membranes were then painted using a glass pipette with a shaved polyethylene pipette tip. A 100 mV potential was applied across the bilayer, in order to monitor the transmembrane current between two Ag-AgCl electrodes placed in the block reservoirs. After membrane formation, baseline data were collected for a period of 2–3 min. Next, 2 μl of 1 μg/ml gramicidin A solution were injected into the bath and data were further collected for 6–7 min until full membrane conductance was reached. Once the conductance stabilized, 20 μl of LA solution was added to each of the chamber compartments and data were collected for at least 10 min before experiment termination. Control experiments demonstrated that the pH of unbuffered bath remained between 4.5 and 5.5 throughout this procedure. In this range, neither the gramicidin channel conductance nor the fraction of the local anesthetic in the ionized state are sensitive to pH, though the concentration of neutral local anesthetic would vary considerably. The current amplifier used had a feedback resistance of 100 kΩ and the output was sampled at low frequency (10 Hz) and measured manually with a digitizing oscilloscope.

Channel occurrence frequency and average lifetime in many channel membranes. In order to measure the channel occurrence frequency, membranes containing an average of 10–20 conducting channels (rather than millions as in the multi-channel membranes) were utilized. They were formed across an 88 μm aperture fashioned on a disposable polyethylene pipette using monoolein in hexadecane (50 mg/ml). The pipette was melted and shaved at the tip to the specified aperture size, nitred, and press-fit into a Teflon block containing a 3.0 ml reservoir. The block reservoir was filled with 2.97 ml of unbuffered aqueous 1 M KCl solution (pH 5.00) and 1–3 μl of 25 ng/ml gramicidin A in MeOH. A small volume of bath was then drawn into the pipette chamber by gentle suction to adequately bathe a Ag-AgCl electrode suspended in the rear of the pipette. Throughout the experiment, we used 100 mV applied, 100 Hz filter cutoff, and 300 Hz sample frequency and the temperature varied < 1 C° with the range between runs, 24–27°C.

Bilayers were repeatedly broken and reformed between each measurement to assure the even distribution of the lipid, the gramicidin, and after its addition, the local anesthetic. Neither the saline/local anesthetic solution nor the lipid/peptide solution was exchanged in the process. To avoid contamination or modification of the lipid/peptide solution on the tip of the teflon brush used to paint the bilayer, the brush was carefully stored between bilayers. Care was taken to minimize the amount of lipid on the pipette aperture so that the bilayer diameter was uniformly close to 88 μm. Each bilayer was maintained for approx. 1 min, then broken by the application of a one volt pulse. The next bilayer was then reformed immediately with the teflon brush. During this process, the membrane current was monitored continuously. When the channel formation rate was found to be reasonably stable, 30 μl of LA or control solution was added to the bath with the membrane broken, the chamber stirred for 1–2 min, and the membrane formation procedure continued. In order to allow equilibration of the bilayer thickness and area, and of the peptide and local anesthetic distribution in the bilayer, the first 8 s of the current record for each bilayer was excluded from analysis. In the subsequent 45-s intervals, single-channel currents were counted by detecting stepwise positive current transitions using an AC trigger double-threshold algorithm [7]. The algorithm allowed reliable

analysis of membranes with 15 or more simultaneously conducting channels.

Single-channel current and lifetime measurements. Experimental conditions were the same as for the many-channel experiments described above except that 1–3 μl of 10 ng/ml gramicidin A was added and the membrane current was filtered at 30 Hz and sampled at 100 Hz. Single-channel current amplitudes were analyzed as described previously [7]. Single-channel lifetimes were measured by matching each positive transition with the most probable corresponding negative transition, randomizing when ambiguous. All measurements were made at room temperatures between 24.5 and 27.5°C.

For single-channel conductance and lifetime measurements, control and test data were collected on each of three separate days for each of the three LA's. In each experiment at least 350 channels were measured for both the control and test cases. Care was taken to assure that channels occurred at a low frequency so that no more than two or three channels were conducting simultaneously.

It was important to assure that an effective dose of LA was present for the single channel measurements, and that single channel properties such as the channel lifetime are not inadvertently subject to systematic errors. A 'washout' design was difficult in this case because of the fragility of the lipid bilayer, so a test/control design was used instead. For each experimental session, single channel properties were measured in several bilayers on the same day (approx. 100 channels/bilayer). Half of the session was done with LA present, and half done without as the control, in arbitrary order. The chamber was stirred prior to the formation of a bilayer. All experiments were performed at room temperature, which was measured before and after control and test measurements and remained within 1 C° for each experiment. Standard channels were distinguished from mini's using criteria previously published [7] and were defined as those falling within approx. 2 S.D. of the mode value. Mini's were those channels with conductances less than the standard channel range. To test if the standard channel conductance decreased with LA's present, the one-tailed t-test was used. The channel lifetimes were binned in 0.25-s intervals and fitted with one- and two-exponential functions. For the two-exponential model, we considered the second term insignificant unless $0.9 > a_2 > 0.1$ and $\tau_2 < 0.5\tau_1$ or $\tau_2 > 2.0\tau_1$ where a_2 is the probability of channel occurrence in the second population, τ_2 is the mean lifetime for the second population, and τ_1 is the mean lifetime of the first population (for which the probability of occurrence is $a_1 = 1 - a_2$). If these criteria were satisfied, an F-test [6] was performed using the chi square values of the curve-fits for the two models to determine if the two-exponential fit was significantly different. Unless the F-test results indicated a significantly better fit (using a significance level of $P < 0.05$), the one-exponential model was taken as appropriate.

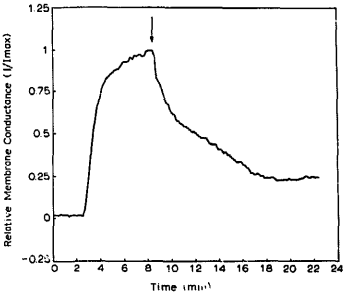


Fig. 1. Decrease in multi-channel membrane conductance from dibucaine. At $t = 2$ min, membrane conductance rises after addition of 2 μl of 1 μg/ml gramicidin A to 1.0 M KCl bath. The vertical arrow at 8 minutes indicates addition of 20 μl of drug to both chamber compartments to yield a concentration of 0.1 mM dibucaine in the bath. 1 M KCl, 100 mV, monoolein/hexadecane.

Results

Multi-channel membrane conductance

Fig. 1 depicts a typical result from a series of experiments with 0.1 mM dibucaine in 1.0 M KCl. Normalized membrane conductance is plotted as a function of time. At 2 min, the membrane conductance increases sharply and plateaus after 2 μl of 1 μg/ml gramicidin A is added to the solution bath. The vertical arrow in the figure at 8 min indicates the addition of 20 μl of dibucaine to each chamber compartment to yield a final concentration of 0.1 mM drug in solution bath. Fig. 1 illustrates a 77.1% drop in membrane conductance after addition of dibucaine. For all drugs, full anesthetic effect was consistently achieved within 10 min after LA addition. A decrease in membrane conductance of at least 25% was consistently seen during experiments, but the exact extent of conductance drop was poorly reproducible. For the other two drugs, maximal membrane conductance drops of 32.9% and 46.5% were observed for 1.0 mM butacaine and 0.5 mM tetracaine, respectively. While the extent of membrane conductance drops were variable, the previously reported concentrations consistently produced significant levels of inhibition to gramicidin A-induced membrane conductance.

Channel occurrence frequency and average channel lifetime in many-channel membranes

Fig. 2 shows membrane current traces before (top) and after (bottom) addition of dibucaine to the bath in a many channel experiment. In the top trace, 308 channels were observed to turn on during the 45 s observation period (of which 40 s are shown here), yielding a channel occurrence rate of 410/min. After addition of dibucaine, 40 channels were observed in the 45 s period, yielding an occurrence rate of 53/min. The occurrence rates for all bilayers measured in this fashion are plotted in fig. 3. The triangles represent a control experiment and demonstrate the variance in the occurrence frequency from bilayer to bilayer due to variability in the peptide distribution in the bilayer, the area of the bilayer, the Poisson process counting error, etc. After sufficient evidence of stable occurrence frequency, local anesthetic dissolved in 30 μl methanol, or as a control, methanol alone, was added to the chamber and vigorously stirred. In Fig. 3, methanol without dibucaine was added to the chamber at $t \sim 24$ min with no significant effect (triangles). For the test case, the aliquot of methanol, containing 0.1 M dibucaine (which, after dilution, yielded a concentration of 1 mM) was added at $t \sim 15$ min, and resulted immediately in a sharp decrease in occurrence rate. In the first eight bilayers prior to the addition of dibucaine, the channel occurrence frequency averaged 449 ± 66 (S.D.)/min. After the addition, the occurrence frequency in the next 17 bilayers was 103 ± 41/min. For two other identical experiments, the occurrence frequency ratios (before/after dibucaine) were 544 ± 203 ($n = 11$)/52 ± 29 ($n = 21$) and 333 ± 54 ($n = 9$)/239 ± 120 ($n = 11$).

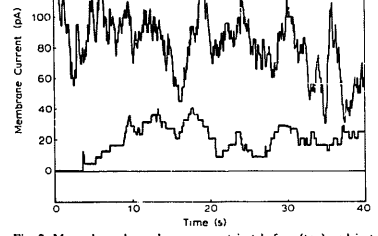


Fig. 2. Many-channel membrane current just before (top) and just after (bottom) addition of 1 mM Dibucaine to the bath. 1 M KCl, 100 mV, monoolein/hexadecane, low-pass cutoff 100 Hz, 25°C.

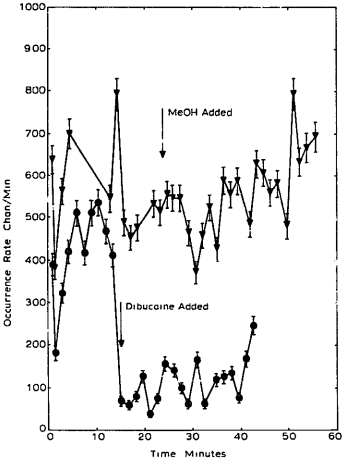


Fig. 3. Rate of gramicidin channel occurrences in lipid bilayers over a period of 60 min. Each point represents the channel frequency in a separate bilayer during the 45-s period prior to the time the point is plotted. Triangles represent the rate of occurrences in a control experiment where 1% methanol was added to the bath at 24 min. Circles represent the experiment where 1 mM Dibucaine was added (in addition to 1% methanol) at 15 min. Error bars represent ± 1 S.D. of the mean channel occurrence rate calculated from the counting error for the observation period with the assumption that channel occurrence was a stationary Poisson process. 1 M KCl, 100 mV, monoolein/hexadecane, low-pass cutoff 100 Hz, 24–27°C.

The same experiments allowed the average channel lifetime, λ, to be estimated using the equation:

$$\lambda = \frac{\bar{I}_m}{iF} \tag{1}$$

where $\bar{I}_m$ is the mean membrane current during the 45 s interval, i is the average single-channel current, and F is the channel occurrence frequency. The averaged channel lifetimes, λ, that correspond with the occurrence rate groups in the experiments reported above were (before/after dibucaine): 1.99 ± 0.11 s ($n = 10$)/4.67 ± 1.04 s ($n = 18$); 3.15 ± 0.21 s ($n = 13$)/3.45 ± 0.83 s ($n = 14$); and 2.87 ± 0.24 s ($n = 11$)/3.90 ± 0.62 s ($n = 11$). Thus for these experiments the estimated average channel lifetime significantly increased or remained unchanged, rather than decreasing upon the addition of dibucaine.

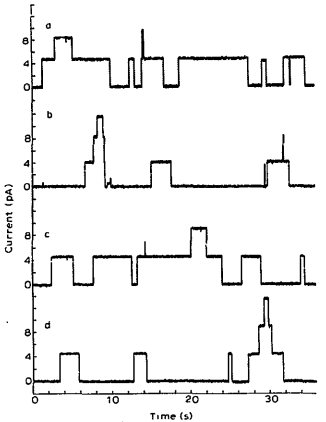


Fig. 4. Gramicidin A single-channel currents for control channels and channels equilibrated for 10 min with local anesthetics. Tics on the horizontal axis are at 2-s intervals; vertical axis tics are at 2-pA intervals. (a) Drug-free, (b) in 0.1 mM dibucaine, (c) in 1.0 mM butacaine, and (d) in 0.5 mM tetracaine. 1 M KCl, 100 mV, monoolein/hexadecane, low-pass cutoff 30 Hz, 26–27°C.

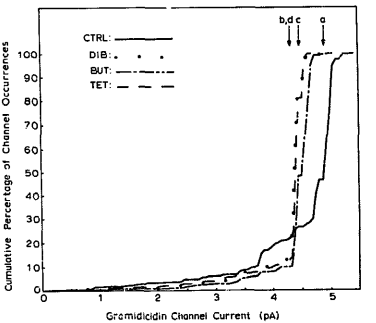
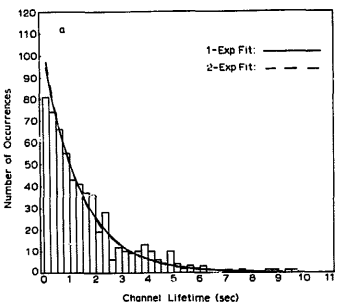


Fig. 5. Cumulative distribution plot of single-channel conductances. The percentage of single-channel currents with amplitudes less than or equal to x-axis values are plotted at 0.061 pA intervals. The control population is denoted with a solid line. The LA-exposed populations are: 0.1 mM dibucaine (dotted line), 1.0 mM butacaine (dashed/dotted line), and 0.5 mM tetracaine (dashed line). Arrow a marks the control mean standard channel conductance; b, c, and d mark the standard channel conductance in dibucaine, butacaine, and tetracaine. The total channel population size and percentage of minis in the populations are listed in Table I. 1 M KCl, 100 mV, monoolein/hexadecane, low-pass cutoff 30 Hz, 26–27°C.



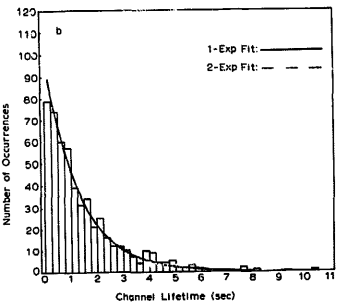


Fig. 6. Channel lifetime histograms for a channel population exposed (a) or not exposed (b) to 0.5 mM tetracaine. Histogram bins are 0.25 s in width. The solid curve represents curve-fitting with a one-exponential model computed to be for a: $y_1(t) = 686 \cdot \exp(-t/1.432)\{1-\exp(-\Delta t/1.432)\}$. The dashed curve is the fit with the two-exponential model, $y_2 9t) = 219 \cdot \exp(-t/1.06)\{1-\exp(-\Delta t/1.06)\} + 467 \cdot \exp(-t/1.61)\{1-\exp(-\Delta t/1.61)\}$ For b, the solid curve is the fit with the one-exponential model computed to be, $y_1(t) = 686 \cdot \exp(-t/1.345)\{1-\exp(-\Delta t/1.345)\}$. The dashed curve is the fit with the two-exponential model, $y_2(t) = 97 \cdot \exp(-t/1.209)\{1-\exp(-\Delta t/1.209)\} + 589 \cdot \exp(-t/1.369)\{1-\exp(-\Delta t/1.369)\}$. Refer to Table I, Tetracaine Set 3 for the total number of channels and percentage of minis in the population. 1 M KCl, 100 mV, monoolein/hexadecane, low-pass cutoff 30 Hz, 26–27°C.

Single-channel amplitude and lifetime

Sample single-channel currents recorded for control and LA-doped channels are depicted in Figs. 4, a–d. Currents were measured in 1.0 M KCl with 100 mV membrane potential applied at a room temperature of 26–27°C. Fig. 4a illustrates control channel currents, while Figs. 4b, c, and d depict test channels collected for 0.1 mM dibucaine, 1.0 mM butacaine, and for 0.5 mM tetracaine, respectively. Most of the channels in these traces are standard channels; however, mini channels are seen at 3 and 28 s in trace a and 8, 9, and 29 s in trace b. From the traces, it is seen that control and all test channels were similar in appearance. No induction of flicker blocks was observed with the introduction of LA's. Under the data collection and filtering conditions used, flicker blocks of more than 6.5 ms would yield a signal which would cross a 50% threshold [27].

In the presence of LA's, the surviving channels have a conductance distribution similar to controls. The standard channel peak is shifted to a lower conductance, as shown by comparison of the positions of the vertical sections of curves b, c, and d in the cumulative distribution in Fig. 5 to that of the control, curve a, but also represents a larger proportion of the total population (~ 90% vs. 73%). Thus the mean conductance of the surviving channels is unchanged: in Table I the μ_{tot} ratio (test/control) averaged 0.98.

Fig. 6 illustrates results of one- and two-exponential curve-fitting of test (a) and control (b) populations in tetracaine Set 3. The solid line represents a one-exponential curve-fit and the two-exponential fit is marked with a dashed line. The differences between one- and two-exponential curve-fits for Fig. 6 and in all 18 data sets in Table I are barely resolvable. Reduced chi square values for the one- and two-exponential curve-fits are 1.4 and 1.4, respectively, for Fig. 6a and 0.5 and 0.6 for Fig. 6b.

Because a one-exponential model sufficiently fit the lifetime histograms, it was reasonable to compare control and LA-influenced standard channel mean lifetimes. Single channel lifetimes for the same experiment sets are summarized in Table II. Mean single channel lifetime (μ) and standard deviation (σ), for control and test groups are listed in the same manner as in Table I. The bottom row of Table II gives the probability that the mean lifetime for the test case is lower than that of the control case, based on a one-tailed t-test.

TABLE I

Mean conductance comparison of control and LA-influenced standard channels

Dibucaine (0.1 mM)	Set 1		Set 2		Set 3	
	control	LA	control	LA	control	LA
μ_{std} (pA)	4.593	4.044	4.481	4.017	4.989	4.480
σ_{std} (pA)	0.1702	0.1189	0.1013	0.1159	0.1176	0.074
μ_{tot} (pA)	3.885	3.926	4.140	3.717	4.578	4.307
N_{tot}	421	421	733	733	368	368
% Mini's	41.6	10.2	29.5	22.2	30.2	14.4
Temp. (°C)	24.5	24.5	25.0	24.5	26.5	26.0
P	$P < 0.01$		$P < 0.01$		$P < 0.01$	
Butacaine (1.0 mM)	**Set 1**		**Set 2**		**Set 3**	
	control	LA	control	LA	control	LA
μ_{std} (pA)	4.359	4.356	4.870	4.578	4.972	4.944
σ_{std} (pA)	0.0812	0.1229	0.1221	0.1038	0.1152	0.886
μ_{tot} (pA)	4.109	4.198	4.670	4.457	4.666	4.703
N_{tot}	384	384	506	506	626	626
% Mini's	21.1	12.8	14.2	9.7	17.9	31.8
Temp. (°C)	25.0	25.0	26.0	27.0	27.0	27.0
P	$P < 0.01$		$P > 0.05$		$P > 0.05$	
Tetracaine (0.5 mM)	**Set 1**		**Set 2**		**Set 3**	
	control	LA	control	LA	control	LA
μ_{std} (pA)	4.867	4.495	4.980	4.555	4.951	4.502
σ_{std} (pA)	0.1044	0.1757	0.0923	0.0956	0.0713	0.0595
μ_{tot} (pA)	4.645	4.381	4.742	4.410	4.638	4.326
N_{tot}	515	515	496	495	686	686
% Mini's	13.8	7.8	19.6	14.9	23.5	13.8
Temp. (°C)	26.0	26.0	26.5	27.0	27.0	27.0
P	$P < 0.01$		$P < 0.01$		$P < 0.01$	

TABLE II

Mean lifetime comparison of control and LA-influenced standard channels

Dibucaine (0.1 mM)	Set 1		Set 2		Set 3		Mean	
	control	LA	control	LA	control	LA	control	LA
μ (s)	2.368	2.631	2.414	2.950	2.704	2.854	2.495	2.812
σ (s)	2.770	3.134	2.496	2.804	2.457	2.366	0.1488	0.1337
P							$P > 0.05$	
Butacaine (1.0 mM)	Set 1		Set 2		Set 3		Mean	
	control	LA	control	LA	control	LA	control	LA
μ (s)	3.097	2.628	2.711	2.096	1.796	2.589	2.535	2.438
σ (s)	3.012	2.683	2.593	1.988	2.208	2.776	0.5456	0.2431
P							$P > 0.05$	
Tetracaine (0.5 mM)	Set 1		Set 2		Set 3		Mean	
	control	LA	control	LA	control	LA	control	LA
μ (s)	2.760	3.481	2.393	2.166	1.434	1.580	2.196	2.409
σ (s)	2.828	3.503	2.344	2.515	1.430	1.544	0.5590	0.7949
P							$P > 0.05$	

Channel dissociation rates defined by the reciprocal of the mean channel lifetime were not significantly reduced by the presence of LA's.

Discussion

A binding receptor mechanism has been used to explain local anesthetic inhibition of current through ion channels like the Na and ACh channel [13,21]. The LA receptors have usually been characterized as hydrophobic sites in or about the channel which bind LA's to occlude the channel and/or enhance channel inactivation. The present study was intended to examine whether LA's block gramicidin dimer channels in the same way.

A reduction in mean single channel current or flicker blocks would have indicated binding to the entry of the channel. We observed a significant decrease in standard single channel conductance amplitude. However, LA dosages yielding a 25–75% decrease in overall transmembrane conductance did not affect the average conductance of surviving channels nor induce flicker blocks. Therefore the primary inhibitory mechanism cannot be an occluding type.

We also considered whether channel lifetimes were reduced, e.g. due to enhanced channel inactivation. Conceivably, LA could thicken the bilayer or permanently bind to gramicidin monomers before dimerization and cause channels with abbreviated lifetimes. A thickened bilayer would have a single population of channels with reduced lifetime [14]. Channels with peptide strongly bound would have produced a second exponential term in the lifetime distribution. However, the channel lifetime distributions were unaffected by local anesthetics in the single channel experiments (Fig. 6, Table II) and mean lifetimes were only modestly affected in the many-channel membrane experiments. The facts that channel conductance and lifetimes were not reduced are consistent with the observation that the inhibition by LA is primarily due to a lower channel occurrence frequency. In particular, the lifetime results argue against the possibility that the equilibrium constant for intramembrane dimer formation is altered by a general mechanism such as membrane swelling, which should reduce channel lifetime as well as occurrence frequency.

LA's probably reduce the channel occurrence frequency by enhancing gramicidin aggregation or solubility in the aqueous phase which reduces the proportion of gramicidin molecules in the bilayer. The long time-course of inhibition (approx. 10 min in Fig. 1) and the poor reproducibility of multi-channel inhibition would seem to support this mechanism. Another possibility is that LA's may alter membrane properties at the lipid/water interface reducing the membrane adsorption of gramicidin monomers.

Acknowledgments

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References

1 Adams, P.R. (1977) J. Physiol. (London) 268, 291–318.
2 Akaike, N., Ito, N., Nishi, K. and Oyama, Y. (1982) Br. J. Pharmacol. 76, 37–43.
3 Andersen, O.S. (1984) Annu. Rev. Physiol. 46, 531–548.
4 Balgavy, P. and Ondrias, K. (1982) in Liquid Crystals and Models

of Biological Membranes, Smolenice, Czechoslovakia, Abstracts, p. 94.
5 Bean, B.P., Cohen, C.J. and Tsien, R.W. (1983) J. Gen. Physiol. 81, 613–642.
6 Bevington, P.R. (1969) in Data Reduction and Error Analysis for the Physical Sciences, Ch. 10, pp. 187–203, McGraw-Hill, New York.
7 Busath, D.D., Andersen, O.S. and Koeppe, R.E., II (1987) biophys. J. 51, 79–88.
8 Carmeliet, E., Morad, M., Van der Heyden, G. and Vereecke, J. (1986) J. Physiol. (London) 376, 143–161.
9 Dawson, D.C., Van Driessche, W. and Helman, S.I. (1988) Am. J. Physiol. 254, 165–174.
10 Elliot, J.R., Haydon, D.A. and Hendry, B.M. (1987) Pflügers Arch. Eur. J. Physiol. 409, 589–595.
11 Elliot, J.R., Haydon, D.A. and Hendry, B.M. (1987) Pflügers Arch. Eur. J. Physiol. 409, 596–600.
12 Hille, B. (1977) J. Gen. Physiol. 69, 475–496.
13 Hille, B. (1977) J. Gen. Physiol. 69, 497–515.
14 Hladky, S.B. and Haydon, D.A. (1972) Biochim. Biophys. Acta 274, 294–312.
15 Koeppe, R.E. and Weiss, L.B. (1981) J. Chromatogr. 208, 414–418.
16 McLaughlin, S. (1975) in Progress in Anesthesiology, Vol. 1, Molecular Mechanisms of anesthesia (Fink, B.R., ed.), pp. 193–220, Raven Press, New York.
17 Meyers, V.B. and Haydon, D.A. (1972) Biochim. Biophys. Acta 274, 313–322.
18 Moczydlowski, E., Uehara, A., Guo, X. and Heiny, J. (1986) Ann. NY Acad. Sci. 479, 269–292.
19 Narahashi, T., Anderson, N.C. and Moore, J.W. (1967) J. Gen. Physiol. 50, 1413–1428.
20 Narahashi, T., Frazier, D.T. and Moore, J.W. (1972) J. Neurobiol. 3, 267–276.
21 Neher, E. and Steinbach, J.H. (1978) J. Physiol. (London) 277, 153–176.
22 Ondrias, K., Balgavy, P., Stolc, S. and Horvath, L.I. (1983) Biochim. Biophys. Acta 732, 627.
23 Ondrias, K., Berczi, A., Misik, V. and Stolc, S. (1986) Stud. Biophys. 115, 17–22.
24 Oyama, Y., Sadoshima, J.-I., Tokutomi, N. and Akaike, N. (1988) Brain Res. 442, 223–228.
25 Richards, N.W. and Dawson, D.C. (1986) Am. J. Physiol. 251, 85–89.
26 Ruff, R.L. (1977) J. Physiol. (London) 264, 89–124.
27 Sigworth, F.J., Urry, D.W. and Prasad, K.U. (1987) Biophys. J. 52, 1055–1064.
28 Strichartz, G.R. (1973) J. Gen. Physiol. 62, 37–57.
29 Urry, D.W., Goodall, M.C., Glickson, J.D. and Mayers, D.F. (1971) Proc. Natl. Acad. Sci. USA 68, 1907–1911.
30 Vassilev, P.M., Hadley, R.W., Lee, K.S. and Hume, J.R. (1986) Am. J. Physiol. 251, H475–H480.
31 Yamamoto, N. and Kasai, M. (1982) J. Biochem. 92, 477–484.