Small iminium ions block gramicidin channels in lipid bilayers

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ABSTRACT Guanidinium and acetamidinium, when added to the bathing solution in concentrations of ~0.1M, cause brief blocks in the single channel potassium currents from channels formed in planar lipid bilayers by gramicidin A. Single channel lifetimes are not affected indicating that the channel structure is not modified by the blockers. Guanidinium block durations and interblock times are approximately exponential in distribution. Block frequencies increase with guanidinium concentration whereas block durations are unaffected. Increases in membrane potential cause an increase in block frequency as expected for a positively charged blocker but a decrease in block duration suggesting that the block is relieved when the blocker passes through the channel. At low pH, urea, formamide, and acetamide cause similar blocks suggesting that the protonated species of these molecules also block. Arginine and several amines do not block. This indicates that only iminium ions which are small enough to enter the channel can cause blocks in gramicidin channels.

INTRODUCTION

Several transmembrane ion channels have been found to undergo transient "flicker" blocks when exposed to specific chemical agents. Flicker blocks could be defined as interruptions in single channel currents lasting long enough to be detectable given the frequency limitations of the current-measuring device, i.e. > ~10 μs (Yellen, 1984; Heinemann and Sigworth, 1988). For instance, flicker blocks are caused by local anesthetics in acetylcholine receptor channels (Neher and Steinbach, 1978), by Na^+, Cs^+, and Sr^+ in anomalous rectifier potassium channels (Fukushima, 1982), by Na^+ in Ca^{2+}-activated K^+ channels (Yellen, 1984) and by Cd^{2+}, Mg^{2+}, and Ca^{2+} in calcium channels (Lansman et al., 1986). However, the molecular structure of these channels is unknown, so the physical basis of the flicker blocks remains speculative.

Gramicidin is an uncharged helical peptide which dimerizes to form cation-selective channels in lipid bilayer membranes (Andersen, 1984). It is a useful model of permeation for some membrane channels because it exhibits the properties of a cation-selective, single-filing pore (Hladky and Haydon, 1972; Urban et al., 1980). When exposed to 1 M formamide, it undergoes high frequency blocks which are not detectable as flicker events, but which cause an increase in single channel noise (Heinemann and Sigworth, 1988). Divalent cations (e.g., 0.1–1.0 M Ca^{2+} or Ba^{2+}) also reduce single channel potassium currents, but without inducing flicker blocks (Bamberg and Läuger, 1977).

We report here that a group of small iminium ions cause flicker blocks in the gramicidin A channel. This finding provides an opportunity to identify the structural basis for flicker blocks for this model channel. One member of the group, guanidinium, is particularly interesting because its behavior with gramicidin may serve as a model for the interactions of other guanidino blocking agents such as tetrodotoxin (Ritchie and Rogart, 1977), amiloride (Bentley, 1968), and nonylguanidinium (Farley et al., 1981; Morello et al., 1980) with other channels in cell membranes. The interactions of the iminium ions with gramicidin channels will hopefully be useful in illuminating the geometry of the narrow channel. A preliminary report of this work has appeared (Busath et al., 1988).

METHODS

Lipid bilayers were formed on the aperture of a polyethylene pipette inserted into the rear of a teflon chamber (Busath and Szabo, 1988). 5% glyceryl monoolein (NuChek Prep, Inc., Elysian, MN) dissolved in *n*-hexadecane (Burdick and Jackson, American Laboratory Supply, Babson Park, MA) was painted on the pipette aperture and allowed to thin spontaneously. Solutions were made using distilled water purified to >18.2 MΩ-cm with a Barnstead NANOpure II system (VWR Scientific, San Francisco, CA) and were filtered just before use with a 0.2-μm Nalge filter (Fisher Scientific, Pittsburgh, PA). Potassium chloride (Fisher Scientific) was roasted at 600°C for 3 h before use. Formamide-spectro grade, urea, glycine (all from Fisher Scientific),

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acetamide, formamidine hydrochloride, guanidine hydrochloride, acetamidine hydrochloride, methylamine hydrochloride, ethylamine hydrochloride, dimethylamine hydrochloride, hydrazine monohydrochloride (all from Aldrich Chemical Company, Milwaukee, WI), 2-aminoethanol, arginine, guanidinoacetic acid (all from Sigma Chemical Company, St. Louis, MO), decyl-guanidine (a gift of Dr. Chris Miller, Brandeis University), and amiloride (Merck Chemical Division, Rahway, NJ) were used without purification. Alternative sources of guanidinium hydrochloride (Sigma Chemical Co. and Fisher Scientific) and formamide (Spectro grade, Eastman Kodak Company, Rochester, NY) were tested and yielded identical results. For one experiment, guanidinium was recrystallized from 85% acetone, 15% methanol, and tested for channel blocking ability. ^{14}C-guanidinium spiked crystals had constant specific activity after one recrystallization. The frequency and duration of block found with 0.5 M recrystallized guanidinium chloride (in 1.0 M KCl at 100 mV) did not differ appreciably from the results reported here for the unpurified salt. Gramicidin A was purified from gramicidin D (ICN Nutritional Pharmaceuticals, Cleveland, OH) by high performance liquid chromatography using the method of Koeppe and Weiss (1981).

To obtain a channel occurence frequency low enough for the observation of single channels, we added 2 μl methanolic (VWR Scientific or Fisher Scientific, HPLC grade) gramicidin A to the 2-ml saline solution bathing the membrane, yielding a final gramicidin concentration of ~10 pg/ml or ~5 pM. The transmembrane current was measured using an amplifier and filter built by Tatsuo Iwazumi (presently of the University of Calgary School of Medicine, Calgary Alta, Canada), low-pass filtered with a fourth-order Bessel filter using a cutoff frequency of 3 kHz, and digitized with 12-bit resolution at 10,000 samples/s for analysis with a Masscomp computer. Single channel currents using gramicidin A purified in our lab were similar to those reported by other labs (Busath and Szabo, 1981; Hladky and Haydon, 1972).

Standard channels were identified as those with conductances falling within the main gaussian peak (Busath et al., 1987). The block duration was estimated as the time between the last data point preceding the block in the fully open state to the last point in the fully blocked state. The interblock time was the time between the last point in the fully blocked state and the last data point preceding the next block in the fully open state, excluding the events immediately after channel opening and preceding channel closing. Because the channel lifetime was ~1000-fold longer than the block duration, channel closures were distinguishable from blocks by their durations. The mean and standard deviation of n block or interblock times, each taken from > 3 standard channel in 1–3 different membranes are reported as the mean ± SD (n). The standard errors are usually < 10%. For guanidinium, the block frequency was approximated as the inverse of the average interblock time, which is appropriate when the average block duration is small relative to the time between blocks.

RESULTS

Fig. 1 shows traces of channel current in the absence (*a*) and presence (*b*) of 0.5 M guanidinium. It is evident in Fig. 1 *b* that guanidinium induces frequent blocks of channel current. With an expanded time scale (Fig. 1 *c*), the clusters of blockades are found to be distinct, well-resolved interruptions of channel current. It can be seen in Fig. 1 *b* that those blockades lasting long enough

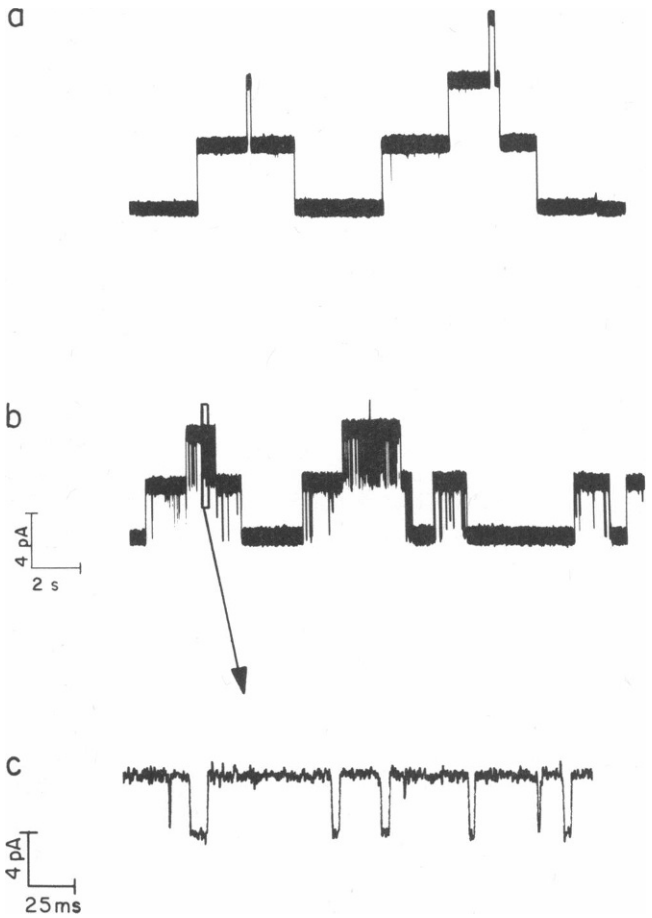


FIGURE 1 Membrane current after the addition of gramicidin A (to 5 pM) into the adjacent compartments which contained 1.0 M KCl (*a*) or 1.0 M KCl + 0.5 M guanidine HCl (*b* and *c*). Upward deflections represent increases in membrane conductance. Low-pass filter cutoff: 3 kHz; sample rate: 10,000/s. Applied potential: 100 mV; 23°C.

to be fully resolved usually appeared to be complete, i.e., no residual channel current flows during a blockade.

The addition of guanidinium has a moderate effect on the conductance of the unblocked state of the gramicidin channel. At a concentration of 0.5 M, guanidinium chloride reduces the standard channel unblocked conductance to 36 pS (vs. 44 pS without guanidinium; 1.0 M KCl; 100 mV; 21–23°C). This effect is expected due to a decreased potassium mobility (Andersen, 1983*b*) and activity and may also reflect fast guanidinium interactions with the channel mouth. Guanidinium has no effect on the mean channel lifetime which we measured to be 2.63 s (n = 235) without guanidinium vs. 2.65 s (n = 221) with 0.5 M guanidinium chloride. This indicates that guanidinium does not perturb the hydrogen bonds connecting the two peptide monomers at the center of the channel nor the channel-length/membrane-

thickness ratio because these factors strongly affect average channel lifetimes (Hladky and Haydon, 1972). Figs. 2, *a* and *b* are histograms of block durations and time between blocks, respectively. Both variables are approximately exponential in distribution as demonstrated by the curves, suggesting that blocking and unblocking are associated with a simple binding-unbinding process.

Average block and interblock times at four different concentrations are given in the first four lines for guanidinium in Table 1. The block rate increases as the guanidinium concentration is increased (Table 1). The rise is approximately linear with guanidinium activity. The guanidinium block duration is unaffected by increased guanidinium concentration, as expected for noncooperative blocking. With no guanidinium present, there is a low block frequency, 2.1 blocks/s, which is due to spontaneously occurring blocks (Ring, 1986; Sigworth and Shenkel, 1988).

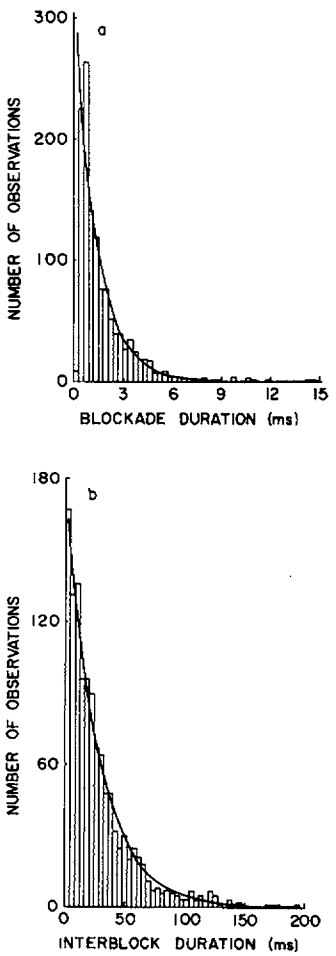


FIGURE 2 Histograms of block durations (*a*) and times between blocks (*b*) for blocks induced by 0.5 M guanidine HCl (Table 1, guanidine, 3rd line). Experimental conditions as in Fig. 1. Smooth curves represent an exponential distribution with a characteristic time equal to the observed mean.

Formamide, urea, acetamide, and acetamidine also cause transient blockades in conductance. These are similar in appearance to those induced by guanidinium. Block and interblock times for these blockers are given also in Table 1. From the first four lines of data for urea, it can be seen that the blockade frequency increases with increasing urea concentration similar to guanidinium.

The block rate increases with membrane potential for both guanidinium and urea. This can be seen by comparing in Table 1 the last three lines for guanidinium and the third and fifth lines for urea. The results for guanidinium are also illustrated in Fig. 3 which shows single channel current traces obtained at increasing membrane potentials. In the 200-mV trace, the flicker blocks are shorter and more frequent than at lower potentials. For guanidinium, the block frequency in Table 1 increases 2.62-fold between 100 and 150 mV (at 200 mV the block frequency is not as accurate because of the short block duration), which could be interpreted to mean that the transition state on the reaction coordinate pathway to blocking is located 48% down the electric gradient from the bath. A similar increase is seen with urea (compare lines 3 and 5 for urea in Table 1), but cannot be calculated accurately because of the very high frequency and short duration of the urea blocks.

Blocks get shorter with increasing membrane potential (Table 1 and Fig. 3), indicating that blocker exit occurs at the end opposite to blocker entry. If the charged iminium plugged the channel entrance but could not pass, increased voltage would lengthen the blocks; if it penetrates, increased voltage should speed its passage. The guanidinium block duration in Table 1 is reduced 1.6-fold when the applied potential is increased from 100 to 150 mV.

For formamide and urea, the block frequency increases with increased $[H^+]$ (Table 1) suggesting that blocks are only induced by these molecules when they are protonated. The block durations were not affected by the pH, suggesting that once one of these molecules is blocking the channel, its charge is irrelevant or insensitive to the pH outside the channel. In the absence of urea or formamide, blocks are not observed in the pH range 1–3 (with or without added 1.0 M KCl) indicating that protonation of the peptide or lipid are not responsible for the blocks reported in Table 1. The blocks were too short in duration to allow accurate measurement of the block frequency. Therefore, the pH dependence of the block frequency has not been analyzed quantitatively.

Under similar conditions of membrane potential, the block frequency sequence from Table 1 is formamide >

TABLE 1 **Iminium block kinetics**

	pH*	Vm	Conc.‡	Block time	Interblock time
		mV	*M*	*ms*	*ms*
Formamide	1.0	100	0.1	0.25 ± 0.09 (642)	7.03 ± 6.74 (640)
"	2.0	"	"	0.30 ± 0.22 (231)	55.08 ± 70.71 (221)
Urea	1.0	100	0.1	0.59 ± 0.55 (264)	10.93 ± 10.59 (260)
"	"	"	0.2	0.60 ± 0.45 (160)	6.36 ± 6.58 (157)
"	"	"	0.5	0.59 ± 0.49 (170)	3.52 ± 2.83 (169)
"	"	"	1.0	0.61 ± 0.50 (177)	1.62 ± 1.49 (178)
"	"	200	0.5	0.31 ± 0.16 (149)	1.05 ± 0.90 (146)
"	2.0	100	0.1	0.53 ± 0.43 (232)	67.65 ± 73.78 (225)
Acetamide	1.0	100	0.1	0.40 ± 0.30 (206)	113.93 ± 108.97 (144)
Guanidine	5.7	100	0.1	2.22 ± 2.21 (84)	153.86 ± 204.79 (78)
"	"	"	0.2	2.33 ± 1.92 (139)	102.93 ± 109.28 (127)
"	"	"	0.5	2.23 ± 2.63 (180)	52.95 ± 45.15 (174)
"	"	"	1.0	2.62 ± 3.15 (290)	38.72 ± 40.27 (291)
"	"	150	0.1	1.38 ± 2.07 (64)	72.49 ± 64.91 (62)
"	"	200	"	0.97 ± 0.57 (127)	45.94 ± 45.67 (125)
Acetamidine	5.0	100	1.0	6.26 ± 8.34 (42)	494.32 ± 503.62 (27)

*Titrated with HCl.
‡Blocker concentration added to symmetric 1.0M KCl, 23–25 C.

urea > acetamide (all at pH 1) > guanidine > acetamidine. The blockade durations approximately follow the inverse sequence. Some small organic cations, formamidine, ethanolamine, methylamine, ethylamine, dimethylamine, and hydrazine (each at concentrations of 0.1 M), and large molecules, glycine, arginine (0.1 M), decyl-guanidine (20 μM), guanidinoacetic acid (50 mM), and amiloride (500 μM) did not induce detectable blockades.

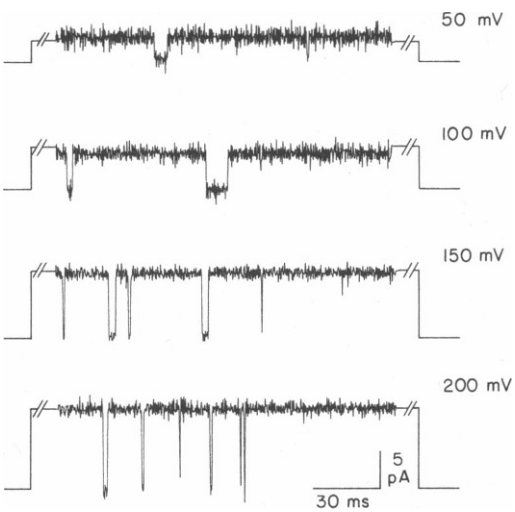


FIGURE 3 Single standard channel currents using 1.0 M KCl + 1.0 M guanidine HCl at increasing applied membrane potentials. (Standard channels are those having the most typical conductance value). The start and end of each single channel current record are shown diagrammatically. Blocks get shorter and more frequent as voltage is increased. 10,000/s sample rate; 23°C.

DISCUSSION

The structures, sizes, and pK_a's of the small iminium ions are shown in Table 2. The blockers all have a trigonal shape and at least one NH_2. It is reasonable to assume that to pass through the channel lumen they would orient like a rectangle passing through a cylinder. The long side of the rectangle would align parallel to the cylinder axis. The fit of the rectangle in the cylinder would depend on whether the rectangle's width were less than the cylinder's diameter. To measure the widths of the molecules in Table 2, CPK models (Ealing, Inc., South Natick, MA) of the molecules were viewed on edge and rotated to present the shortest width which was measured with calipers and converted to nanometers. The measurements were made with (H-B) or without (no H-B) H-bonding hydrogens on all nitrogens and oxygens. This approach ignores the thickness of the flat molecule and utilizes the van der Waals dimensions of the atoms rather than the hard core dimensions (Busath et al., 1988) but gives a useful comparison of the relative sizes of the molecules. The blocker sizes are all greater than the proposed diameter of the static pore interior, ~0.4 nm (Urry, 1971; Busath et al., 1988) but they may be accommodated by librations of the peptide backbone

(Popov and Lipkind, 1979; Venkatachalam and Urry, 1984).

The pH dependence of the urea- and formamide-induced block rate suggests that urea and formamide must also be positively charged when blocking. The pK_a's of these molecules, listed in Table 2, is near zero. Although limitations in the frequency response of the amplifier prevented accurate measurements of the block rates for these two molecules, it appears that the urea block frequency is approximately proportional to the concentration of the charged species estimated from the pK_a using the Henderson–Hasselbach equation. X-Ray and neutron crystallography studies demonstrate that the carbonyl oxygen in urea becomes protonated at low pH (Harkema and Feil, 1969; Worsham and Busing, 1969). This probably also occurs with formamide and acetamide, given their structural similarity to urea. The molecules can then assume the same resonance structures as guanidinium, formamidinium, and acetamidinium, including formation of partial double bonds between carbon and nitrogen.

Formamidinium behaves differently from the other iminium ions in Table 2. It has an iminium group and it should be big enough to block because formamidium, which is the same size, causes blocks. However, we were unable to detect blocks using formamidinium. Eisenman et al. (1976) have reported that the permeability of gramicidin to formamidinium is very high, higher than to K^+. Thus formamidinium's channel passage time would be too short (<30 ns) to cause detectable blocks. The reason for the high permeability of formamidinium is unclear.

Next, we assume that the observable blocks represent most of the guanidinium passages and evaluate the results following the method outlined by Woodhull (1973) and extended to apply to flicker blocks by Yellen (1984). Although there is debate about the merit of transition-state rate theory for ion transport in gramicidin channels (Finkelstein and Andersen, 1981; Jakobsson and Chiu, 1987; Cooper et al., 1988), it appears to be appropriate for guanidinium transport because the energy barriers to entry and exit must be $\gg 2$ kT. The arguments that follow are illustrated by the free energy diagram in Fig. 4.

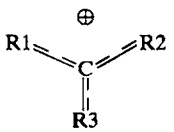
If the entry rate for a guanidinium ion is as low as the block rate, there must be a large barrier to channel entry. The barrier to formamidinium entry must be fairly small. The formamidinium single-channel conductance is similar to that of sodium, ~14 pS at 1 M, 100 mV (Xian-Zheng Jin and David Busath, unpublished results), and therefore its transport rate is near the diffusion-limited rate for a pore of diameter 4 A (Urban et al., 1980). Therefore the barrier to guanidinium entry can be estimated as:

$$\Delta G_1 = -RT \ln \frac{k_{guan}}{k_{form}}, \quad (1)$$

where R is the gas constant, T absolute temperature, k_{guan} is the entry rate for guanidinium, estimated as the block rate, and k_{form} is the entry rate for formamidinium, estimated as the transport rate. The voltage dependence of the entry step is assumed to be the same for both ions and the measurements to be made at equivalent membrane potentials and permeant ion concentrations. If k_{form} is 4.5×10^7/s and k_{guan} is 25.8/s, ΔG_1 is 8.6 kcal/mol (Fig. 4).

The voltage dependence of the block rate (Table 1) is approximately e-fold per 52 mV, suggesting that the entry barrier for the blocker would be located ~48% through the electric potential drop, i.e., half way through the channel. This seems unlikely because the process of entry would be expected to occur over a few Angstroms in the first 10% of the potential drop. A small part of the voltage dependence could be attributed to access resistance of the bath (~6%; Andersen, 1983*b*) and increased local guanidinium concentration due to charging of the bilayer by guanidinium (~1.5%; Andersen,

TABLE 2 Iminium characteristics

					Width (*nm*)		
		R1	R2	R3	H-B	no H-B	pKa (ref)
	Formamidium	H	OH	NH_2	0.44	0.47	−0.5*
	Formamidinium	H	NH_2	NH_2	0.45	0.50	11.2†
	Uronium	NH_2	OH	NH_2	0.53	0.57	0.1§
	Acetamidium	CH_3	OH	NH_2	0.54	0.56	−1.4§
	Guanidinium	NH_2	NH_2	NH_2	0.54	0.58	13.6§
	Acetamidinium	CH_3	NH_2	NH_2	0.55	0.59	12.4§

*Albert and Sergeant, 1984.
†Harkema and Feil, 1969.
§Worsham and Busing, 1969.

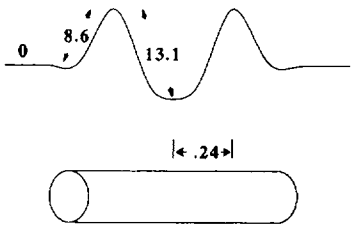


FIGURE 4 Free energy profile estimated based on the kinetics of guanidinium blocks. The labels are free energies in kcal/mol (see text for method). The cylinder below represents the gramicidin channel and is marked to show the approximate distance to the peak of the exit barrier which is based on the voltage dependence of the block durations.

1983*a*). Perhaps there is some other aspect of the guanidinium block process which is highly voltage dependent.

The energy profile between the two entry barriers is suggested by the duration of the blocks and its dependence on membrane voltage. We assume that guanidinium exit from the channel is blocked by the same barrier referred to above. The height of the exit step, ΔG_2, could be estimated from the average block duration using Eyring rate theory (Eyring et al., 1949; Woodbury, 1971; Läuger, 1973; Hille, 1975; for caveats see Cooper et al., 1988) with the equation:

$$\frac{1}{\tau} = f\frac{kT}{h}e^{-\Delta G_2/RT}, \qquad (2)$$

where f, the transmission coefficient is taken arbitrarily as 0.1, and k, T, and h are Boltzmann's constant, absolute temperature, and Planck's constant. The height of the barrier with no applied potential is calculated by extrapolating logarithmically from the block durations in Table 1 at 100 and 150 mV to 0 mV. Using τ (0 mV) = 4.7 ms, ΔG_2 is calculated from Eq. 2 to be 13.1 kcal/mol. The observation reported in Table 1 that the block duration decreases by a factor of 1.6 with an increase in potential of 50 mV would be interpreted to indicate that the electric field drops 24% between the binding site in the channel and the peak of the exit barrier. An alternative interpretation of the block duration is that the ion diffuses slowly over a series of smaller barriers as it passes through the channel.

These entry and exit barriers compare favorably with potential energy barriers computed using energy minimization (Busath, D., B. Turano, M. Pear, K. Gaffney, and M. Karplus, manuscript submitted for publication) and free energy barriers computed using molecular dynamics (Pear and Busath, 1990).

In summary, the new gramicidin blockers described above are all iminium ions which apparently are small enough to fit into and pass through the channel yet are large enough or of sufficient affinity to bind on the way through for detectable periods. Because of their rigid structures and tight fit in the channel lumen, the blockers could provide a valuable new probe of the internal geometry of gramicidin channels.

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REFERENCES

Albert, A., and E. P. Serjeant. 1984. The Determination of Ionization Constants, A Laboratory Manual. Chapman and Hall, London. 151–152.

Andersen, O. S. 1983*a*. Ion movement through gramicidin A channels. Interfacial polarization effects on single channel currents. *Biophys. J.* 41:135–146.

Andersen, O. S. 1983*b*. Ion movement through gramicidin A channels. Studies on the diffusion-controlled association step. *Biophys. J.* 41:147–165.

Andersen, O. S. 1984. Gramicidin channels. *Annu. Rev. Physiol.* 46:531–548.

Bamberg, E., and P. Läuger. 1977. Blocking of the gramicidin channel by divalent cations. *J. Membr. Biol.* 35:351–375.

Bentley, T. J. 1968. Amiloride: a potent inhibitor of sodium transport across the toad bladder. *J. Physiol.* 195:317–330.

Busath, D., and G. Szabo. 1981. Gramicidin forms multi-state rectifying channels. *Nature (Lond.).* 294:371–373.

Busath, D., and G. Szabo. 1988. Low conductance gramicidin A channels are head-to-head dimers of $\beta^{6.3}$-helices. *Biophys. J.* 53:689–695.

Busath, D. D., O. S. Andersen, and R. E. Koeppe II. 1987. On the conductance heterogeneity in membrane channels formed by gramicidin A. A cooperative study. *Biophys. J.* 51:79–88.

Busath, D., G. Hemsley, T. Bridal, M. Pear, K. Gaffney, and M. Karplus. 1988. Guanidinium as a probe of the gramicidin channel interior. *In* Transport Through Membranes: Carriers, Channels and Pumps. A. Pullman, J. J. Jortner, B. Pullman, editors. Kluwer Academic Publishers, Boston (Norwell, MA 02061) 187–201.

Cooper, K. E., P. Y. Gates, and R. S. Eisenberg. 1988. Diffusion theory and discrete rate constants in ion permeation. *J. Membr. Biol.* 106:95–105.

Decker, E. R., and D. G. Levitt. 1988. Use of weak acids to determine the bulk diffusion limitation of H^+ conductance through the gramicidin channel. *Biophys. J.* 53:25–32.

Eisenman, G., S. Krasne, and S. Ciani. 1976. Further studies on ion selectivity. *In* Ion Selective Electrodes and Enzyme Electrodes in Medicine and in Biology. M. Kessler, L. Clark, D. Lubbers, I. Silver, and W. Simon, editors. Urban and Schwarzenberg, Munich. 3–22.

Eyring, H., R. Lumry, and J. W. Woodbury. 1949. Some applications of modern rate theory to physiological systems. *Rec. Chem. Prog.* 10:100–114.

Farley, J. M., J. Z. Yeh, S. Watanabe, and T. Narahashi. 1981. Endplate channel block by guanidine derivatives. *J. Gen. Physiol.* 77:273–293.

Finkelstein, A., and O. S. Andersen. 1981. The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport. *Membr. Biol.* 59:155–171.

Fukushima, Y. 1982. Blocking kinetics of the anomalous potassium rectifier of tunicate egg studied by single channel recording. *J. Physiol.* 331:311–331.

Harkema, S., and D. Feil. 1969. The crystal structure of urea nitrate. *Acta Crystallogr.* B25:589–591.

Heinemann, S. H., and F. J. Sigworth. 1988. Open channel noise IV. Estimation of rapid kinetics of formamide block in gramicidin A channels. *Biophys. J.* 54:757–764.

Hille, B. 1975. Ionic selectivity of Na and K channels of nerve membranes. *In* Membranes—A Series of Advances. G. Eisenman, editor. Marcel Dekker, Inc., New York. 3:255–323.

Hladky, S. B., and D. A. Haydon. 1972. Ion transfer across lipid membranes in the presence of Gramicidin A. 1. Studies of the unit conductance channel. *Biochim Biophys. Acta.* 274:294–312.

Jakobsson, E., and S.-W. Chiu. 1987. Stochastic theory of ion movement in channels with single-ion occupancy. Application to sodium permeation of gramicidin channels. *Biophys. J.* 52:33–45.

Jordan, P. C. 1988. A molecular dynamics study of cesium ion motion in a gramicidin-like channel. Structural and energetic implications. *In* Transport Through Membranes: Carriers, Channels and Pumps. A. Pullman, B. Pullman, and J. Jortner, editors. Kluwer Academic Publishers, Dordrecht, Netherlands. 237–251.

Koeppe, R. E. II, and L. B. Weiss. 1981. Resolution of linear gramicidins by preparative reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 208:414–418.

Lansman, J. B., P. Hess, and R. W. Tsien. 1986. Blockade of current through single calcium channels by Cd^{2+}, Mg^{2+}, and Ca^{2+}. Voltage and concentration dependence of calcium entry into the pore. *J. Gen. Physiol.* 88:321–347.

Läuger, P. 1973. Ion transport through pores: a rate-theory analysis. *Biochim. Biophys. Acta.* 311:423–441.

Morello, R. T., T. Begenisich, T. Woubalem, and J. K. Read. 1980. Interaction of nonylguanidine with the sodium channel. *Biophys. J.* 31:435–440.

Neher, E., and J. H. Steinbach. 1978. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol.* 277:153–176.

Pear, M., and D. Busath. 1990. Free energy profile for iminium transport by gramicidin: guanidinium vs. formamidinium. *10th International Biophysics Congress, Abstracts.* 393:P5.5.7.

Popov, E. M., and G. M. Lipkind. 1979. Conformational state and mechanism of the functioning of gramicidin A. *Mol. Biol. (USSR).* 13:363–376.

Ring, A. 1986. Brief closures of gramicidin A channels in lipid bilayer membranes. *Biochim. Biophys. Acta.* 856:646–653.

Ritchie, J. M., and R. B. Rogart. 1977. The binding of saxitoxin and tetrodotoxin to excitable membranes. *Rev. Physiol. Biochem. Pharmacol.* 79:1–50.

Sigworth, F. J., and S. Shenkel. 1988. Rapid gating events and current fluctuations in gramicidin A channels. *Curr. Top. Membr. Transp.* 13:113–130.

Urban, B. W., S. B. Hladky, and D. A. Haydon. 1980. Ion movements in gramicidin pores. An example of single-file transport. *Biochim. Biophys. Acta.* 602:331–354.

Urry, D. W. 1971. The gramicidin A transmembrane channel: a proposed $\pi_{(L,D)}$ helix. *Proc. Natl. Acad. Sci. USA.* 68:672–676.

Urry, D. W., K. U. Prasad, and T. L. Trapane. 1982. Location of monovalent cation binding sites in the gramicidin channel. *Proc. Natl. Acad. Sci. USA.* 79:390–394.

Venkatachalam, C. M., and D. W. Urry. 1984. Theoretical analysis of gramicidin A transmembrane channel: II. Energetics of helical librational states of the channel. *J. Comput. Chem.* 5:64–71.

Woodbury, J. W. 1971. Eyring rate theory model of the current-voltage relationships of ion channels in excitable membranes. *In* Chemical Dynamics: Papers in Honor of Henry Eyring. J. Hirschfelder, editor. Wiley, New York. 601–617.

Woodhull, A. M. 1973. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* 61:687–708.

Worsham, J. E. Jr., and W. R. Busing. 1969. The crystal structure of uronium nitrate (urea nitrate) by neutron diffraction. *Acta Crystallogr.* B25:572–578.

Yellen, G. 1984. Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. *J. Gen. Physiol.* 84:157–186.