that measurements of channel conductances and their activation enthalpies provide two useful independent tests for any theory.

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ELECTROSTATIC MODELS OF THE GRAMICIDIN AND THE DELAYED RECTIFIER POTASSIUM CHANNEL

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I have treated an ion-pore former-membrane–water system as an electrostatic problem involving a set of distinct dielectric phases. The effect that reasonable variation of the system's physical and electrical structure has on the energy barrier to ion permeation and on the voltage profile generated by an applied potential is used to show that: (*a*) a parameter-free model is consistent with gramicidin channel properties that are clearly dependent on coulombic interactions; and (*b*) electrostatic considerations severely limit the range of possible structures for the delayed-rectifier potassium channel.

GRAMICIDIN A

The gramicidin channel is treated as a uniform cylinder of length 2.6 nm with binding sites 0.25 nm from each end (see reference 1), embedded in membranes of variable width. The aqueous solutions and the water within the pore form a dielectric phase with $\epsilon = 80$. The pore former and the lipid form another dielectric phase with $\epsilon = 2$. To compensate for dielectric shielding by the polar groups lining the pore interior, the pore's effective electrical radius is 0.25 nm (2), larger than its physical radius. Membrane dipole potentials are estimated from surface potential measurements of lipid monolayers on water (3). A mean charge distribution for gramicidin is determined from atomic coordinates found by computer modeling of the β-helical structure (4) and partial charge parameters representative of atoms in amino acids (5). It is equivalent to a uniform dipole density of 8×10^{-12} C m^{-1} smeared out on a cylinder of radius 0.37 nm; the negative end of the dipoles points inward. The model suppresses local variation.

The total electrical potential due to the ion image, the membrane potential and the pore-former charge distribution is calculated for membranes of variable width by an extension of my previous work (2, 6). This establishes the electrostatic potential energy profile and the additional repulsive energy when both binding sites are occupied. The electrostatic contribution to the energy barrier at the channel center, ϵ^*, to the energy barrier to translocation, ϵ_t, and the ratio of the binding constants for single and double occupancy, K_1/K_2, are plotted in Fig. 1 as functions of membrane width for phosphatidylcholine (PC) and glyceryl monooleate (GMO) bilayers.

In addition to electrostatic energy, the total potential contains a term reflecting differences in the solvating ability of water and of polar groups lining the pore. In bilayers ~5 nm wide the activation barrier to Na^+ conductance is ~31 kJ/mol^{-1} in PC (7) and ~20 kJ/mol^{-1} in GMO (8). Because ϵ^*, for a 5-nm GMO membrane, is ~28 kJ/mol^{-1}, the local solvation energy must be < -8 kJ/mol^{-1}. If binding in the channel is slightly exothermic (1, 9) this energy would be more negative.

As solvation energy varies little within the pore[1], ϵ_t is

[1]W. K. Lee and P. C. Jordan. Unpublished results.

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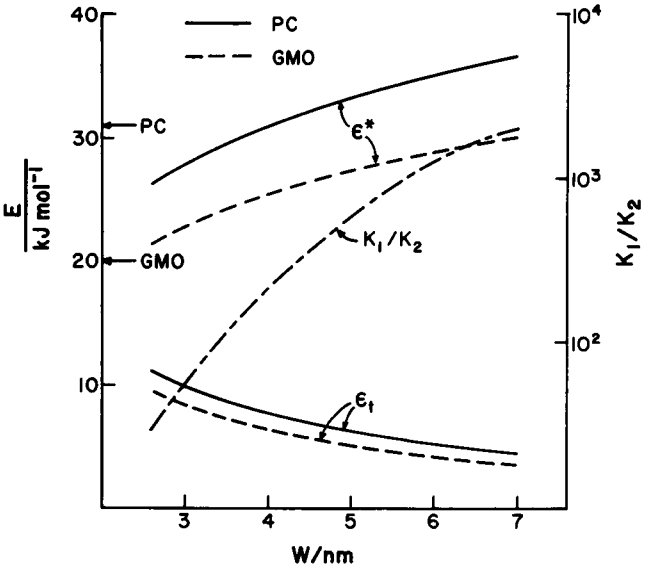

FIGURE 1 Electrostatic contribution to the energy barrier at the channel center, ϵ^*, to the energy barrier to translocation, ϵ_t, and to the binding constant ratio for single and double occupancy, K_1/K_2, for gramicidin channels in PC and GMO membranes of width W. The gramicidin model is described in the text. The arrows indicate the activation energy for channel conductance in PC and GMO.

determined by the electrostatic potential. From Fig. 1, ϵ_t is insignificant: 5–11 kJ/mol^{-1} in PC and 4–10 kJ/mol^{-1} in GMO. It is smaller for thicker membranes, is never more than ~1/2 of the total barrier, and is usually much less. It provides no real impediment to translocation; the potential within the channel is effectively flat.

The binding constant ratio, K_1/K_2, is comparable to that determined experimentally for Na^+, ~100 (9), for membrane widths ≤ 5 nm. Ion-other–image interactions cause small changes in the assumed binding site separation to dramatically alter this ratio. For sites separated by ≤ 1.8 nm the K_1/K_2 ratio is much larger than 100; if they are separated by ≥ 2.4 nm the ratio is far too small. Only distances between 1.95 and 2.25 nm account for the data.

DELAYED RECTIFIER POTASSIUM CHANNEL

Electrostatic considerations yield structural constraints on the physical and electrical geometry of the delayed rectifier potassium channel. The model is illustrated in Fig. 2. The exterior mouth size, the length of the constriction, L, and the effective electrical radius, a_0, are all variable. The length of the right-hand part of the system, Λ, is fixed at 4 nm; for the parameters considered the membrane width varies between 4.8 and 5.5 nm.

The potential profile due to an applied voltage is determined by relating the properties of unsymmetrical channels to those of symmetrical ones.[2] It is accurate if

[2]P. C. Jordan. Unpublished results.

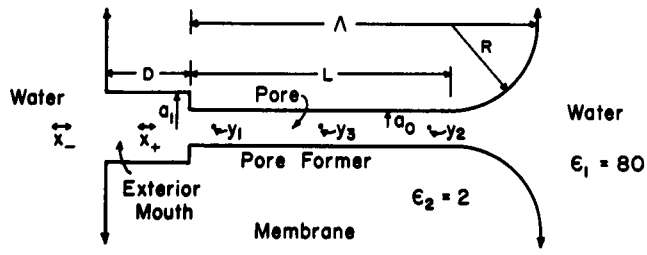

FIGURE 2 Structural model that may be representative of a delayed rectifier potassium channel. The quantities D, a_1, L, and a_0 are all variable while $\Lambda = L + R = 4$ nm. Water and the water molecules in the exterior mouth and the pore have $\epsilon = 80$. The membrane and pore former have $\epsilon = 2$. The effective electrical radius, a_0, may be larger than the physical radius due to the presumed polarity of the pore-former. Blocking is assumed to occur near x_+ or x_- depending upon whether blockers can or can not penetrate the exterior mouth. Tight binding sites are assumed to be at y_1 and y_2 and at y_3 (if there is a third site).

$L/a_0 > 2.5$. Regardless of the parameters chosen—$a_1 = 0.4$ nm or 0.6 nm, $1.5 \leq D/a_1 \leq 3$, $a_0 = 0.15$ to 0.25 nm, $L = 2$ to 4 nm—the potential drop to the vicinity of x_- is only 2/3 of that to the vicinity of x_+. Because blocking by aliphatic quaternary ammonium derivatives with head group diameters between 0.8 and 1.2 nm occurs at the same fractional electrical distance (10, 11), the exterior mouth of the channel must be at least 0.6 nm in radius or the aliphatic chains on the larger blockers must penetrate the structures forming the exterior mouth.

Blocking occurs 15–20% of the electrical distance through the channel (10, 11). For the two-dielectric model to account for this observation, a_0 must be ≳0.2 nm; 0.25 nm is more likely. In addition, D/a_1 must be ~2.5–3. A pore that permits K^+ passage but discriminates against Rb^+ has a physical radius between 0.15 and 0.17 nm. This suggests the pore-former is quite polar; an ϵ of 8–12 is needed for dielectric shielding to accommodate plausible a_0 values. The exterior mouth is ~1–1.2 nm long if $a_1 = 0.4$ nm. These conclusions are independent of the length of the constriction, if $L \geq 2$ nm.

The potassium channel is a multi-ion pore (12). Because its conductance is aqueous-diffusion limited (13), the rate constant for exiting the channel k_-, must exceed the capture rate; this rate, estimated from the total conductance, or the capture rate, is $3 \cdot 10^6$–$2 \cdot 10^7$ s^{-1} (14, 15) in

TABLE 1
UPPER BOUNDS TO THE RATE OF EXITING THE MODEL CHANNEL OF FIG. 2

	Sites occupied*		
L/nm	(1,2,3)	(2,3) or (1,3)	(1,2)
	s^{-1}	s^{-1}	s^{-1}
4	$2 \cdot 10^8$	$5 \cdot 10^8$	$2 \cdot 10^{12}$
3	$6 \cdot 10^3$	$2 \cdot 10^5$	10^{11}
2	0.4	$2 \cdot 10^3$	10^9

*See Fig. 2

0.1 M K^+. An upper bound to k_- is implicit for multiple occupancy to be observed. If two sites are occupied, the extra energy required to bind the second ion is ≤ 0, etc. The single-ion binding energy approximately equals $-$ (total repulsion energy); reaction rate theory then provides an upper bound to k_-. Assuming that the favored tight binding sites are near the end of the constriction (y_1 and y_2 in Fig. 2), and that the third site is in the middle, k_- is given in Table I for $a_0 = 0.25$ nm and various constriction lengths and occupancy possibilities. Because k_- must exceed the capture rate, triple occupancy requires that $L \sim 4$ nm. Double occupancy of the exterior sites is possible if $L \geq 2$ nm. These conclusions are unaltered if another cation is bound externally (at x_+) or if a_0 is decreased to 0.2 nm; the additional repulsion is too small to significantly decrease the bounds to k_-.

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ORIENTATION OF THE DIPHTHERIA TOXIN CHANNEL IN LIPID BILAYERS

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Cytotoxic action of diphtheria toxin (DT; M_r 62,000) requires translocation of its A fragment (DT-A; M_r 21,000) into the cytosolic compartment (Collier, 1975; Pappenheimer, 1977). There DT-A catalyzes transfer of the ADP-ribose moiety of NAD to elongation factor 2, which causes inactivation of the factor and blockage of protein synthesis. The B fragment (M_r 41,000) is responsible for binding DT to the cell surface and is required for transmembrane translocation of DT-A. Various lines of evidence indicate that receptor-bound DT is initially brought into the cell within endosomes and that acidification of the endosomal lumen induces insertion of the toxin into the endosomal membrane and transfer of DT-A to the cytosolic compartment.

Whole toxin has been shown to insert into planar lipid bilayers under acidic conditions, forming ion-conductive channels (Donovan et al., 1981). The amino terminal two-thirds of the B fragment, B45 (M_r 24,000), which alone is capable of forming a channel (d = 18 Å), appears to be necessary for translocation of DT-A across lipid membranes in vitro (Kagan et al., 1981).

Here we report the use of DT ligands to map functional sites of DT with respect to the lipid bilayer. Specifically, we have obtained evidence that a binding site for the dinucleotide ApUp (adenylyl-(3′,5′)-uridine 3′-monophosphate) appears on the *trans* side of the membrane after addition of DT to the *cis* side. Other data suggest that the ApUp site is composed of elements of the NAD site (substrate binding

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