EFFECTS OF DOUBLE-LAYER POLARIZATION ON ION TRANSPORT

ATTICUS H. HAINSWORTH AND S. B. HLADKY
Department of Pharmacology, University of Cambridge, Cambridge, CB2 2QD, England

ABSTRACT It has been proposed that changes in ionic strength will alter the shape of current-voltage relations for ion transport across a lipid membrane. To investigate this effect, we measured currents across glyceryl monooleate membranes at applied potentials between 10 and 300 mV using either gramicidin and 1 mM NaCl or valinomycin and 1 mM KCl. A bridge circuit with an integrator as null detector was used to separate the capacitative and ionic components of the current. The changes in the current-voltage relations when ionic strength is varied between 1 and 100 mM are compared with predictions of Gouy-Chapman theory for the effects of these variations on polarization of the electrical diffuse double-layer. Double-layer polarization accounts adequately for the changes observed using membranes made permeable by either gramicidin or valinomycin.

INTRODUCTION

A lipid membrane separating two bulk aqueous phases behaves as an electrical capacitor. The aqueous solutions correspond to the plates, and the membrane corresponds to the dielectric insulator. When a potential is applied to the membrane a net charge is added to one aqueous phase and an opposite net charge is added to the other. Because these net charges attract each other but can't enter the hydrophobic core of the membrane, the net charge in each aqueous phase accumulates close to the lipid-water interface. Thus, close to the membrane surface, the concentrations of the ions that carry the charges will differ from their values in the bulk of the aqueous phase. At one surface the concentration of cations will increase relative to the bulk concentration and the concentration of anions will decrease, while at the other surface the reverse will occur. The tendency for these ions to diffuse toward or away from the surfaces down their concentration gradients must be balanced by a tendency to migrate driven by a gradient of electrical potential between the membrane surface and the rest of the solution. The thin region of each aqueous phase which becomes polarized as a result of these effects, i.e., the region where the ion concentrations differ from their solution values and there is a gradient of potential, is called the diffuse portion of the electrical double-layer or often just the double-layer. The potential difference across it, which balances the concentration gradients, is known as the (diffuse) double-layer potential. When there are few ions present in the aqueous phases, i.e., the ionic strength is low, the charge accumulation in the double-layer represents a large fractional change in the ion concentrations and the double-layer potential is large. By contrast, at high ionic strength, the charge accumulation represents a negligible difference in the ion concentrations and the double-layer potential is small. At any ionic strength the charge accumulation and the double-layer potential required increase with the capacitance of the membrane.

The applied potential is the sum of the potential difference across the membrane and the double-layer potentials on either side. Thus, when double-layer polarization is significant, it affects both the potential difference across the membrane and the ion concentrations at the membrane surfaces. These changes can affect the rate of ion transport. For low permeant ion concentrations, for which the conductance is proportional to concentration, and for all known current-voltage relations, the predicted effect of the concentration changes to increase the conductance exceeds that of the potential change to decrease it, and the conductance should be greater in the presence of double-layer polarization than in its absence. This increase should become progressively larger with increasing applied potential. Thus, double-layer polarization will tend to make current-voltage relations bend less toward the voltage axis (or more toward the current axis) as the potential is increased.

Addition of inert, i.e., impermeant, nonblocking, and nonadsorbing, ions will have an indirect effect on transport by reducing double-layer polarization. Thus, for low permeant ion concentrations, the predicted effects of adding an inert or supporting electrolyte are to reduce the conductance at each applied potential and to make the current-voltage relation bend more towards the voltage axis.

The use of Gouy-Chapman theory to describe similar effects at the interface between mercury and a salt solution is now well established (Frumkin, 1933 and 1961; Parsons,

A. H. Hainsworth's present address is Department of Physiology, Rush Medical College, Chicago, Illinois.

1961; Delahay, 1965; Vetter, 1967).[1] The first application of the theory to lipid membranes was the successful explanation of the variation of the apparent membrane capacitance with ionic strength (Läuger et al., 1967; Everitt and Haydon, 1968; White, 1973). Walz et al. (1969) then used it to consider the possible effects of double-layer polarization on the transport of lipid soluble ions.

For gramicidin and low permeant ion concentrations, the slope of the current-voltage relation decreases as the potential increases (Hladky and Haydon, 1972; Eisenman et al., 1980). Andersen (1983*a*) has extended these observations to higher potentials using membranes formed from diphytanoyl phosphatidylcholine and *n*-decane. For 10-mM permeant ion concentration, he proposes that the increases in current with potential above 200 mV are a consequence of double-layer polarization and that without it, the current would approach a constant limiting value. He tested this proposal by measuring the channel conductances in the presence of various concentrations of tetraethylammonium chloride (TEACl), which he argued was an inert electrolyte. TEACl did reduce the potential dependence of the high potential currents, but quantitative fitting of theory to the data required an apparent value of the membrane capacitance that was roughly three times larger than the actual membrane capacitance.

Eisenman and Sandblom (1983*a, b;* 1984) have argued that TEA^+ is not inert. They have measured current-voltage relations for Li^+ or Cs^+ as permeant ions with addition of Mg^{++} (as either $MgSO_4$ or $MgCl_2$, 9 mM at low permeant ion concentrations) or TEA^+ (as TEACl, 1 M for all permeant ion concentrations). For intermediate permeant ion concentrations the shape of the current-voltage relation was clearly different when measured with TEA^+ rather than Mg^{++}. They argued that this difference and the increase in conductance at low potentials observed by Andersen when 0.49 M TEA^+ was added both result from a specific interaction of TEA^+ with the gramicidin channels. At low Cs^+ concentrations the shape of the current-voltage curve they measured was the same in the presence of 1 M TEA^+ or 9 mM Mg^{++}. The lack of any difference between solutions of such different ionic strengths led them to conclude (Eisenman and Sandblom, 1983*a*) that double-layer polarization effects are negligible at least for membranes formed from glyceryl monooleate. Eisenman and Sandblom (1984) concluded that the potential dependence observed by Andersen at 10 mM results from a potential dependence of the transport step in which an ion leaves the channel.

This study was carried out to determine the effects of double-layer polarization on the shape of current-voltage relations at low ionic strengths. Current-voltage relations have been measured using: low permeant ion concentrations; applied potentials from 10 to 300 mV; membranes made conducting by the addition of either carriers or pores; and various ionic strengths obtained by adding choline chloride or other electrolytes. Using the known value of the membrane capacitance and the measured current-voltage relations at 100 mM ionic strength, the Gouy-Chapman theory for double-layer polarization is used to predict the changes in shape of the current-voltage curves at lower ionic strengths. The agreement is satisfactory.

THEORY

Relation between the Surface and Bulk Potentials

For a constant density of adsorbed surface charges on each surface and arbitrary potential difference between the aqueous solutions, the equilibrium surface potentials for any applied potential can be calculated numerically using the standard Guoy-Chapman theory (see Everitt and Haydon, 1968). For strictly neutral membranes and uni-univalent salts, the equations are simpler and a closed form solution has been given by Läuger et al. (1967).

Glyceryl monooleate membranes are not strictly neutral presumably as the result of impurities (Everitt and Haydon, 1968; White, 1973) and it is likely that the charges on the two surfaces will differ sometimes. At low ionic strengths deviations from neutrality can have an appreciable effect on the potentials at the surfaces. It is thus desirable to have simple expressions that are derived without the restrictions to zero surface charge, symmetry, or uni-univalent salts. From consideration of examples it is clear that with nearly neutral membranes and total ion concentrations of 1 mM or greater, the surface potentials differ from those in the bulk solutions by less than 25mV for applied potentials below 300 mV. With the limitation to small surface potentials, it is shown in the appendix that when the aqueous solutions present on the two sides of the membrane have the same ionic strength, Gouy-Chapman theory leads to:

$$V_m = \frac{C_K}{C_K + 2C_m} V_a + (\sigma^d - \sigma^o)/(C_K + 2C_m) \qquad (1)$$

and

$$V^d + V^o - V'' - V' = (\sigma^o + \sigma^d)/C_K \qquad (2)$$

where the potential difference across the membrane is $V_m = V^d - V^o$, the applied potential is $V_a = V'' - V'$, the potentials at the membrane surfaces are V^o and V^d, the potentials far out in the aqueous phases are V' and V'', the

[1] In these references the change in the diffuse double-layer potential when a potential is applied is treated as an example of overvoltage. Vetter (1967) calls it "charge transfer overvoltage." The diffuse double layer potential is approximately equal to the zeta potential. The compact double layer, discussed in these references, cannot be distinguished in the present study from the membrane itself. Many of the complications encountered at the mercury-water interface can be ignored in the present study because the applied potentials are much smaller.

charge densities on the two membrane surfaces are σ^o and σ^d, the membrane capacitance is C_m, and the capacitance of each double layer is C_K.

The capacity of each of the double layers, i.e., the ratio of the charge accumulated in the diffuse double-layer to the double-layer potential, is equal to that of a slab of water with thickness, K^{-1},

$$C_K = \epsilon\epsilon_o K \tag{3}$$

where $\epsilon = 80$ is the dielectric constant for water, ϵ_o is the permitivity of free space, and the Debye constant, K, is given by:

$$K^2 = \frac{2F^2 I_s}{\epsilon\epsilon_o RT}. \tag{4}$$

$$I_s = (1/2)\sum_i z_i^2 c_i \tag{5}$$

is the ionic strength, z_i is the charge on the i'th type of ion, c_i is its concentration in the aqueous phases, F is the Faraday constant, R is the gas constant, and T is the absolute temperature.

In the experiments reported here $C_m = 5.9$ nF/mm^2 (Fettiplace et al., 1971), and for the values given above, K^{-1} is expressed in nanometers, c is in moles/liter, and C_K is in nF/mm^2

$$K^{-1} = 0.304\, I_s^{-1/2} \tag{6}$$

and

$$C_K = 2{,}340\, I_s^{1/2}. \tag{7}$$

Thus, in a 1 M solution of a uni-univalent salt, $2\,C_m/C_K = 5 \times 10^{-3}$ and the potential across the membrane is almost the same as that applied (Läuger et al., 1967; Everitt and Haydon, 1968; White, 1973). By contrast, for 1 mM, the difference is significant for nearly neutral surfaces, e.g., for $V_m = 200$ mV the total potential difference V_a is predicted to be 232 mV, i.e., there is a potential drop of 16 mV across each double-layer.

Effect of Surface Potentials on Ion Transport for Very Low Permeant Ion Concentrations

It follows from the independence principle (Hodgkin and Huxley, 1952) that for any mechanism that allows individual ions to cross the membrane the current at sufficiently low ion concentrations must obey (see, for example, Hladky, 1979; and compare page 198 in Delahay, 1965)

$$i = zFk(V_m)(c^o \exp[-zFV_m/2RT] - c^d \exp[+zFV_m/2RT]) \tag{8}$$

where c^o and c^d are the concentrations of the permeant ion at the membrane surfaces. The form of the proportionality function, $k(V_m)$, depends upon the conduction mechanism, but, so long as the independence principle applies, $k(V_m)$ does not vary with the current, the fluxes, or the concentrations. For gramicidin A the current at low sodium concentrations tends to reach a limit as the potential is increased (Hladky and Haydon, 1972; Eisenman et al., 1980; Andersen, 1983*a*) and thus at large potentials $k(V_m)$ must decrease with potential almost as fast as $\exp[-zFV_m/2RT]$. (While it is theoretically possible for it to increase, $k(V_m)$ decreases with potential for all known conduction mechanisms that obey independence.)

Provided that the flux of permeant ions is sufficiently small, the ion concentrations and potential at each surface of the membrane will be at equilibrium with the adjacent aqueous phase, i.e.,

$$c^o = c' \exp[-zF(V^o - V')/RT] \tag{9}$$

and

$$c^d = c'' \exp[-zF(V^d - V'')/RT]. \tag{10}$$

The fluxes will be small enough whenever there are no aqueous unstirred layer effects[2] (see section 9-5, Delahay, 1965). Substituting (2), (9), and (10) into (8), the current is given by

$$i = zFk(V_m)\exp[-zF(\sigma^d + \sigma^o)/2RTC_K] \times \{c' \exp[-zFV_a/2RT] - c'' \exp[+zFV_a/2RT]\}. \tag{11}$$

When the surface charges on the two sides are equal, $\sigma^o = \sigma^d$, Eqs. 1 and 11 predict the shape of the current-voltage relation, e.g., $I(V)/I(25\text{ mV})$, at all ionic strengths when the shape at any one ionic strength has been determined empirically. In addition they predict: (*a*) A small symmetrical surface charge will increase or decrease the conductance by the same factor, $\exp[-zF(\sigma^d + \sigma^o)/2RTC_K]$, at all applied potentials. (*b*) An asymmetry in surface charge should affect the current-voltage relation only by shifting the potential dependence of $k(V_m)$ along the potential axis. Unless $k(V_m)$ is a constant independent of V_m a charge asymmetry will produce rectification (cf. Latorre and Hall, 1976). (*c*) For a membrane symmetrical except for the surface charges and permeant ion concentrations, $k(V_m)$ is an even function of V_m, i.e., $k(V_m) = k(-V_m)$. This conclusion follows because $I_2(V)$ must equal $-I_1(V)$ when the charges and concentrations in conditions (*a*) and (*b*) are related by $\sigma_2^d = \sigma_1^o$, $\sigma_2^o = \sigma_1^d$, $c_2'' = c_1'$, and $c_2' = c_1''$.

METHODS

Black lipid films were formed using standard techniques (Fettiplace et al., 1975) from 10 mM glyceryl monooleate in hexadecane. Films ranged

[2]The current through individual gramicidin channels will alter the concentration at the mouth of the pore. However, as discussed by Andersen (1983*a*), these changes will extend only a small distance into the aqueous phase. In the present analysis the small region of the aqueous phase where this access polarization occurs is treated as part of the pore (Hladky, 1984). The rate of ion transfer through these regions is reflected in $k(V_m)$, but not in the values of c^o, c^d, and V_m.

from 0.1 to 1.0 mm in diameter. For experiments with gramicidin the aqueous solution, present on both sides of the membrane, contained 1 mM NaCl together with an impermeant supporting electrolyte, either choline chloride (cholineCl) or $MgSO_4$. The gramicidin was added in very small amounts either in the lipid or to one of the aqueous phases. For experiments with valinomycin, 1 mM KCl was used and the supporting electrolyte was $CaCl_2$ or cholineCl. All experiments were performed at ~20°C.

CholineCl (crystalline grade; Sigma Chemical Co., St. Louis, MO) was taken up in hot ethanol, recrystalized as the solution cooled, washed with acetone, and dried to constant weight in a rotary evaporator. The remaining solid had no odor. It was made up as a 2 M aqueous stock solution and stored at 4°C. $CaCl_2$ (Analar volumetric solution, BDH) and all other salts (analytical reagent grade) were used as purchased. Distilled water was prepared using a commercial still modified by replacing all components containing plasticizers with Teflon. Electrodes were chloridized coiled silver wires. All components which come into contact with the aqueous or lipid solutions were cleaned in dichromate-sulphuric acid mixture.

Current-voltage relations were measured using step changes in applied potential, a bridge circuit (Hladky, 1982), and an integrator as null detector. The output of the null detector was viewed using an amplifier (5A22; Tektronix, Inc., Beaverton, OR) and 5441 variable persistence oscilloscope. As this method has not been described previously, it is set out here in some detail. The circuit is shown schematically in Fig. 1. If the reference arm of the bridge is disconnected and a potential is abruptly applied, the observed response for the current through a conductance, G_{obs}, and capacitance, C_{obs}, in parallel is first a jump in the output of the null detector,

$$V_{nd} = -(C_{obs}/C_d)\, V_a \qquad (12)$$

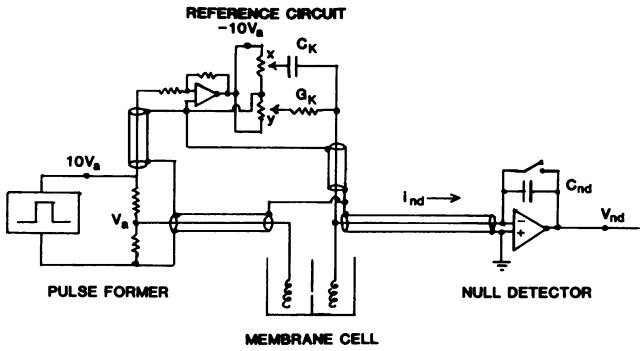

FIGURE 1 Schematic diagram of the apparatus used to measure the current-voltage relations. A sequence of pulses is produced by the pulse former. In the lower arm of the bridge, the potentials are divided by 10 and applied to one electrode of the experimental cell. The other electrode is connected to the summation point of a virtual earth amplifier which serves as a null detector. In the upper arm of the bridge the pulses are inverted using a carefully trimmed wideband amplifier and then applied to model circuits. The current which flows through these circuits is also passed to the null detector. The cables are twisted together to minimize the consequences of the earth loop. The potential at the input of the null detector is always much less than 1 mV, while the current loop into the summation point from the bridge is $i_{nd} = i_m - 10 \times C_K(dV_a/dt) - 10yG_KV_a$, where i_m is the total current through the membrane, x and y are the settings of the potentiometers in the model circuits, $0 < x,y < 1$, C_K is the capacitance of the model capacitor, and G_K is the conductance of the model resistor. The output of the null detector is then $V_{nd} = -\int_0^t i_{nd}/C_{nd}\, dt + V_{nd}(0)$, where $V_{nd}(0)$ is the output just before the sequence of pulses. The null detector is reset to a reference value just before each sequence of pulses.

followed by a steady rate of change in the output

$$dV_{nd}/dt = -V_aG_{obs}/(C_d). \qquad (13)$$

The time course of the initial jump is determined by the time constant for charging the membrane capacitance through any (always small) resistance in series with the model circuit or membrane. Pulses must be long enough to exceed the charging time constant (In all instances discussed here the pulse durations have exceeded this time constant by at least 20-fold.) and short enough that the properties of the membrane do not have time to change and depletion of ions from unstirred layers is negligible.

The factors that limit the accuracy of this instrument when it is used to determine a current-voltage relation are the linearity of the potentiometer in the reference circuit (wirewound, 10 turn, 3 watt, linearity 0.25%), the accuracy of the applied potentials, and the maximum acceptable duration for the pulses. The applied potentials of one sign were set using an AN2570 Panel Meter factory calibrated to within 0.1% (Analogic Corp., Wakefield, MA). Values within 1% were found using other supposedly less accurate digital voltmeters. Pulses of the opposite sign were set so that a pair of pulses of the same length and size but opposite polarity resulted in no net current through model circuits. The timing of the pulses was based on a quartz crystal oscillator (D4030; Digitimer Ltd., Welwyn Garden City, United Kingdom). At each potential, the pulses are applied in pairs of the same magnitude but opposite sign (see Fig. 2 *a*). This procedure largely eliminates cumulative changes in the membrane and aqueous phases. The pulses are typically each 10 ms long, separated by 40 ms. The interval between pairs is sufficiently long that further increase in the interval has no effect on the response.

A tracing of an unbalanced response from an actual membrane is shown in Fig. 2 *b* together with tracings of two further stages of the balancing procedure. With the instantaneous capacitance current balanced, Fig. 2 *c*, the output trace displays two currents, one via the conductance of the membrane, and the other the charging or displacement current corresponding to the changes in membrane capacitance which occur at the new applied potential. These changes in capacitance occur whether or not the membranes are made conducting and continue with a complicated time course for seconds (see, e.g., Benz and Janko, 1976). They correspond to thinning of the membrane (electrostriction) and increases in membrane area. Fortunately, using an integrator as detector, the capacitative and conductive currents are easily separated, because the capacitative currents from before to after the pulse integrate to zero. Thus, the difference in the output voltage between a point just before the pulse is applied and one just after the potential returns to zero is proportional to the charge transfered through the conductance of the membrane. The constant conductance that would yield the same charge transfer is obtained by adjusting the potential applied to a reference conductance. Control membranes have conductances that are below the level of detection of our apparatus using 10 ms pulses, i.e., $<2 \times 10^{-10}$ S. In the experiments for Figs. 4 and 5 the conductances at 25 mV exceeded 2×10^{-9} S.

After the conductance is balanced, Fig. 2 *d*, the residual difference between a point just before the end of the pulse and one just after the beginning represents the capacitance change during the pulse. This change can be determined by balancing first the initial and then the final capacitance. Even though with pulses of 10 ms or less the change in capacitance is always small, usually <1%, the charging current can be an appreciable fraction of the conductive currents that are the object of the investigation. Rectification in the current-voltage relation is immediately apparent as a difference in the output just before and just after the pulse pair. (None of the calibrations or the results reported here are affected by reversing the order of application of the two pulses. Similarly, balancing either the conductance or the capacitance did not affect the balance of the other.)

The conductance of the membrane drifts with time. Thus, it is necessary to refer the conductance at each potential to that at some

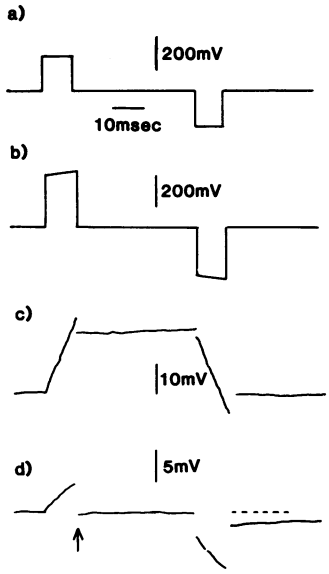

FIGURE 2 A pulse pair and the output from the null-detector at various stages of balancing. Gramicidin, 1 mM NaCl + 99 mM CholineCl, 200 mV applied potential, membrane capacity = 1.75 nF, membrane conductance = 11 nS, upper 3 dB point on the 5A22 amplifier = 10 kHz. (*a*) The pulse sequence. (*b*) The unbalanced response. The height of the jumps is proportional to the capacitance of the membrane. (*c*) After balancing the capacitance at the start of the first pulse and increasing the vertical gain of the oscilloscope. The capacitance can now be read from the setting of the potentiometer, x, and a calibration curve constructed using known capacitors. The total current during the pulse is displayed as the slope of the trace during the pulse. The integral of the current over the pulse appears as the offset of the trace segment just after the first pulse with respect to the initial segment. (*d*) After balancing the integral of the current during the pulse to zero and increasing the gain of the oscilloscope. The average conductance during the first pulse can be determined from the setting y and a calibration curve. The variation in capacitance during the first pulse is indicated by the size of the jump when the potential is returned to zero at the arrow. Rectification is shown by the offset of the final segment of trace with respect to the initial segment.

reference value. In this study conductances are determined at +/− 25 mV, then for the potential of choice and both polarities, and then again at +/− 25 mV. The ratio for each polarity of the test value to the average of the two controls for the same polarity is then calculated. The data presented here are the averages of these ratios for positive and negative pulses. All pulse pairs showing >10% rectification were deleted before calculation of the averages.

In the example given in Fig. 2 the membrane conductance is low and there is no evidence of changes in the aqueous phases, which outlast each pulse. At higher conductances the passage of current during a pulse produces changes that lead to a time-varying current in the reverse direction when the potential is returned to zero. These long-lasting changes represent the onset of diffusion or unstirred layer polarization. At low conductances, paired 10-ms pulses can be used without producing these effects. At higher conductances, shorter pulses (subject to the limitations imposed by the charging time) minimize the impact of diffusion polarization.

RESULTS

The ratio of the conductances at 100 and 25 mV has been measured for a range of conductances that did not display diffusion polarization using paired pulses as described in Methods. Scatter diagrams for the two extreme solutions used with gramicidin are shown in Fig. 3. Data for valinomycin were less scattered and also showed no trend. Over the range of conductances for which data are reported in this paper, the conductance ratios are independent of the membrane conductance.

Potential Dependence of $k(V_m)$

The Gouy-Chapman theory predicts that for 100 mM ionic strength, double-layer polarization will produce only very small effects. Thus, $k(V_m)$ can be determined empirically by assuming that the data obtained for valinomycin or gramicidin at this ionic strength closely reflect the current-voltage relation for the membrane process. (In our calculations, we have assumed that the small effect of double-layer polarization that remains at 100 mM ionic strength is correctly predicted by the model and have used V_m 1.5% less than V_a as predicted by Eq. 1). Any convenient even function of the potential could be used to represent $k(V_m)$ so long as it can be adjusted to fit the data. The fitting function used here is:

$$k(V_m)/k(0) = 1/(1 + b_2\phi_m^2 + b_4\phi_m^4 + b_6\phi_m^6) \quad (14)$$

where

$$\phi_m = FV_m/RT. \quad (15)$$

At room temperature RT/F is ~25 mV. The values of b_2, b_4, and b_6 given in the legends to Figs. 4 and 5 were obtained by forcing exact agreement with the mean values of the ratios $G(50)/G(25)$, $G(100)/G(25)$, and $G(200)/G(25)$ for the data obtained at 100 mM ionic strength.

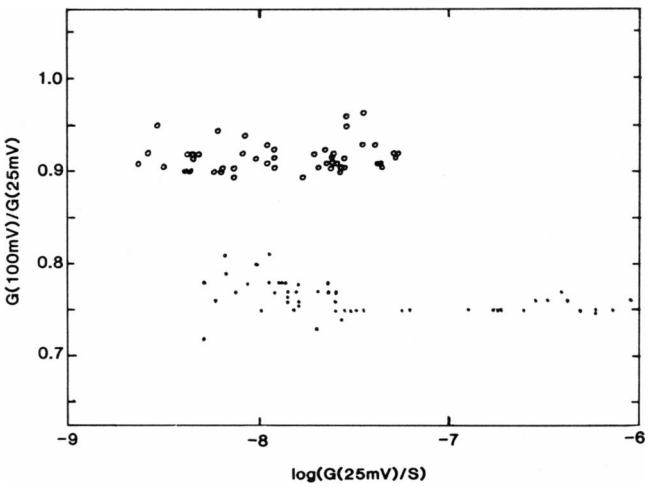

FIGURE 3 Scatter diagram for the ratio of the gramicidin mediated conductance at 100 mV to that at 25 mV as a function of membrane conductance at 25 mV. Upper cluster, 1 mM NaCl. Lower cluster 1 mM NaCl + 99 mM cholineCl. The largest ratio seen in the presence of cholineCl was less than the smallest seen in its absence. Over the range reported, the conductance ratios are independent of the conductance.

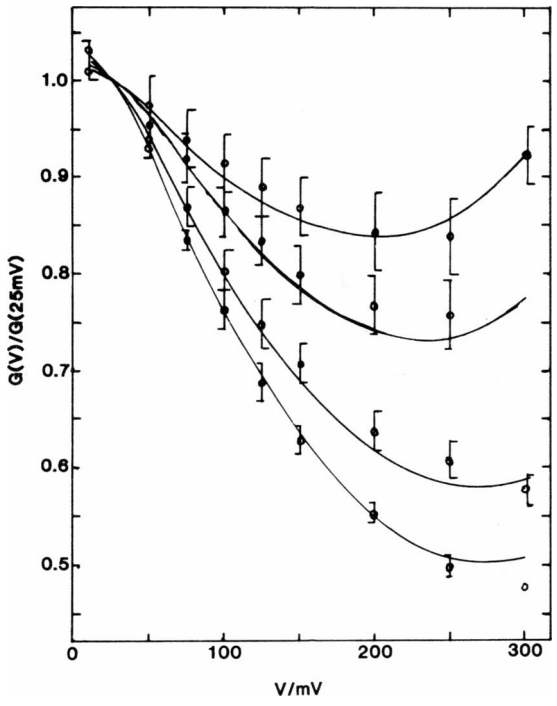

FIGURE 4 The dependence of the conductance ratio on potential and ionic strength for gramicidin and 1 mM NaCl. The ionic strength was increased by the following additions of cholineCl, from the top downwards: 0 mM, 1 mM, 9 mM, and 99 mM. The data are displayed as mean ± SD. The number of observations varies, but for potentials <200 mV, it is always greater than 10 derived from at least two independent experiments performed on different days. The curve for 99 mM added choline$^+$ is used to determine the constants in equation 14: $b_2 = 7.44 \times 10^{-2}$, $b_4 = 9.93 \times 10^{-4}$, and $b_6 = 1.36 \times 10^{-5}$. The curves for 0, 1, and 9 mM added choline$^+$ are calculated with no further adjustable constants using Eqs. 1, 11, and 14.

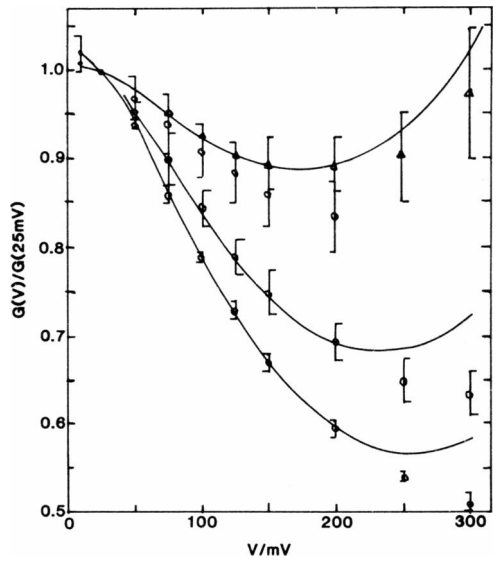

FIGURE 5 The dependence of the conductance ratio on potential and ionic strength for valinomycin and 1 mM KCl. From top to bottom the curves are for ionic strength 1 mM (KCl alone), 5.8 mM (+1.6 mM $CaCl_2$), and 120 mM (+39.6 mM $CaCl_2$). The data are displayed as mean ± SD. The number of observations varies, but for potentials <200 mV it is always greater than 10 derived from at least two independent experiments performed on different days. The mean values of this study are shown as circles; those of C. L. Lawson and S. B. Hladky (unpublished observations) are shown as triangles. Lawson and Hladky's data for lower potentials at 1 mM and for all potentials at 100 mM ionic strength fall within the corresponding error bars for this study. The curve for 39.6 mM added $CaCl_2$ is used to determine the constants in Eq. 14: $b_2 = 6.80 \times 10^{-2}$, $b_4 = 1.08 \times 10^{-3}$, and $b_6 = 1.00 \times 10^{-5}$. The curves for 0 and 5.8 mM ionic strength are calculated with no further adjustable constants using Eqs. 1, 11, and 14.

Current–Voltage Relations at Low Ionic Strength

Using $k(V_m)$ determined from the data for high ionic strength, and the relation between V_m and V_a provided by the Gouy-Chapman theory, Eq. 11 predicts the shape of the current-voltage relations for all other ionic strengths. These curves are compared with data for gramicidin in Fig. 4 and that for valinomycin in Fig. 5. The agreement is satisfactory. The sensitivity of the predictions to the assumed value of the membrane capacity is illustrated in Fig. 6.

As discussed in Methods, data displaying significant rectification are excluded from Figs. 3–6. Irreproducible rectification was observed in many experiments, particularly at low ionic strength. One possible explanation is contamination by surface active materials leading to variations in surface charge. Eqs. 1 and 11, with an asymmetry in surface charge, could describe such data at least qualitatively. (A difference in surface charges of 1 ion per 50 nm^2 could easily produce an offset in $k(V_m)$ of 50 mV and a rectification ratio, $I(+V)/I(-V)$, exceeding 2 for large potentials.) However, the degree of rectification varied over the time required to measure a complete current-voltage relation which precludes quantitative analysis. The origin of the presumed impurities is unknown (compare the discussion in Neher et al., 1978), but the problems were more severe in the experiments with gramicidin.

DISCUSSION

The present experiments have examined the influence of double-layer polarization on currents across black lipid membranes. The membranes were made permeable to cations by the addition of either gramicidin or valinomycin, and the current-voltage relations were measured in the presence and absence of additional impermeant ions. A quantitative description of double-layer polarization for low permeant ion concentrations and sufficiently low applied potentials (at 1 mM ionic strength sufficiently low means less than ~200 mV) can be derived simply from Gouy-Chapman theory and is given as Eqs. 1 and 11. This theory predicts that addition of inert electrolytes will flatten the current-voltage relation by reducing the currents at high potentials. Theory and experiment are compared in Figs. 4–6. The agreement is satisfactory. The changes are sufficiently large that for carriers like nonactin and valinomycin, double-layer polarization will account for a large part of the potential dependence of the rate of

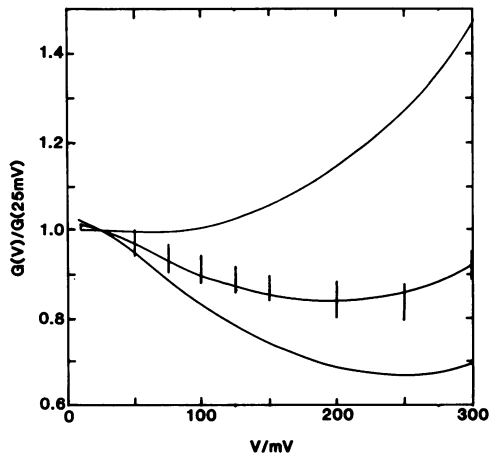

FIGURE 6 The predicted shape of the current-voltage relation for gramicidin and 1 mM NaCl (no added impermeant ions) for different assumed values of the membrane capacitance. These have been calculated using $k(V_m)$ determined from the current-voltage relation for 100 mM ionic strength in Fig. 4 and Eqs. 1 and 11. The curves are, from top to bottom, C_m = 12 nF/mm^2, C_m = 5.9 nF/mm^2, which is the actual capacitance per unit area of the membrane, and C_m = 3 nF/mm^2. The data bars are taken from Fig. 4.

ion-carrier association observed at low ion activities (Hladky, 1974). Furthermore, as proposed by Andersen (1983*a*), polarization in the double-layers will have a major influence on the currents through gramicidin pores at potentials above 100 mV. Fortunately, as discussed elsewhere (Hladky, 1985, 1986; Andersen, 1985), double-layer effects are sufficiently small at lower potentials that they do not compromise those previous interpretations of data for gramicidin which have depended primarily upon the shapes of the conductance-activity relations (Neher et al., 1978; Hladky and Haydon, 1984).

Eisenman and Sandblom (1983*a*) concluded that double-layer polarization was unimportant because 1 M TEA^+ had no significant effect on the current-voltage relation for gramicidin and submillimolar concentrations of cesium. However, they in fact compared 0.1 mM CsCl + 9 mM $MgCl_2$ with 0.5 mM CsCl + 1 M TEACl (1983*b*). Both the calculations and the results presented here show that 9 mM $MgCl_2$ and 1 M TEACl will each eliminate most of the polarization, and thus this comparison cannot be used to argue against the importance of double-layer effects. Nevertheless, Eisenman and Sandblom have demonstrated that for higher concentrations of CsCl, TEA^+ and Mg^{++} do not have the same effect on the current-voltage relations. Furthermore, Andersen's results obtained using gramicidin, diphytanoyl phosphatidylcholine membranes, and 10 mM permeant salts (1983*a;* and Fig. 2) require that TEA^+ at concentrations above 90 mM can alter the currents by some mechanism other than the reduction of double-layer polarization. Andersen suggests that TEACl increases the currents by changing the surface potential of the membrane, while Eisenman and Sandblom (1983*b*) argue that TEA^+ binds to a site near the end of the pore.

Addition of 1 M $CaCl_2$ produces a block of gramicidin pores, which is stronger at higher potentials (Bamberg and Läuger, 1977; Urban, 1978). Because such different impermeant salts as $CaCl_2$ and TEACl apparently have effects on ion currents through the pore which cannot be explained by changes in double-layer polarization, it is almost certain that such effects play some part in the changes in shape of the current-voltage relations reported here. There are, however, several arguments that this part is minor under the conditions investigated. First, $MgCl_2$ blocks gramicidin channels less well than $CaCl_2$ and in glyceryl monooleate membranes even the effects of $CaCl_2$ are much less marked at 100 mM than at 1 M (Bamberg and Läuger, 1977; Urban, 1978). Second, double-layer polarization accounts adequately for the results with valinomycin as well as with gramicidin. Alternative effects of impermeant ions are unlikely to be the same for both. Third, if effects outside the double-layers were the explanation for the changes seen in Figs. 4 and 5, then different impermeant salts should be effective at different ionic strengths. In preliminary experiments to test this point with gramicidin (Lawson, C. L., and S. B. Hladky, unpublished observations), cholineCl, TEACl, tris(hydroxymethyl)aminomethane chloride, $MgCl_2$, and $MgSO_4$ at ionic strengths below 100 mM all produced similar effects consistent with those reported here. Data for 24.25 mM $MgSO_4$ fall within the bars for 99 mM cholineCl shown in Fig. 4. Thus, at these relatively low concentrations, both corresponding to an ionic strength of 100 mM, these two very different salts have the same effect on the shape of the current-voltage relation as expected if they act to remove double-layer polarization. For valinomycin, a similar comparison between $CaCl_2$ and cholineCl gave equally good agreement.

In the present study the capacitance of the unmodified membrane, 5.9 nF/mm^2, has been used in the Gouy-Chapman theory to predict the effects of double-layer polarization for both gramicidin and valinomycin. Andersen (1983*a*) has argued that the correct capacitance to use with gramicidin is a local capacitance in the region of the pore, C_m^*, that theoretically C_m^* should be larger than C_m though by an unspecified amount,[3] and that by two

[3]If the absolute value of the potential difference between the solution and the position occupied by an ion about to enter the pore is greater than $|V^d - V''|$, then C_m^* should be greater than C_m. The change in potential near the mouth of the pore may differ from that near the surface of the unmodified membrane because the applied field induces different dielectric polarization in the region of the pore and in the surrounding membrane. An estimate of the size of this effect can be provided by considering the pore to be a right cylindrical plug with radius r_p (0.4 nm), length d (3 nm), and dielectric constant, ϵ_p spanning a membrane with dielectric constant ϵ_h (2). For simplicity the thickness of the membrane is also taken to be d. The effect of making the length of the pore less than the membrane thickness should be similar to that of increasing the dielectric constant of the pore. The aqueous solutions are represented as perfect conductors separated from the membrane by charge-free dielectric layers

methods of calculation from the experimental data C_m^* is 10–15 nF/mm^2. The difference between this value and the 5.9 nF/mm^2 that fits the present data (see Fig. 6) may be a consequence of the different types of membrane used in the two studies. However, also note that Andersen's calculations of C_m^* were based on assumptions about the currents that would be seen if double-layer polarization were absent. Thus, in one method it was assumed that the current-concentration relation at high potentials would be linear from 10 to 100 mM,[4] and in the other it was assumed that the current-potential relations for the same range of concentrations would be flat at high potentials.[5] The only experimental data directly supporting these assumptions were obtained in the presence of high concentrations of TEACl. Because TEACl can affect the current in a manner not accounted for in the theory, further experiments are required to determine C_m^* for gramicidin in diphytanoyl phosphatidylcholine plus *n*-decane membranes.

The present results argue strongly that changes in ionic strength per se can substantially alter the shape of current-voltage relations measured at low ionic strength. Because these effects of changing ionic strength are adequately explained by the theory of double-layer polarization for two different mechanisms of ion transport and for very different putative inert salts, we conclude that these effects are the result of changes in double-layer polarization and that the size of these effects is, at least for our systems, correctly predicted using the Gouy-Chapman theory and the independently measured capacitance of the membrane.

APPENDIX

Gouy-Chapman theory starts from the Poisson equation

$$d^2V/dx^2 = -\rho/\epsilon\epsilon_o \tag{16}$$

where the charge density is given by:

$$\rho = \sum_i z_i F c_i \tag{17}$$

with the sum running over all species of ion present. Boltzman relations are then used to express the concentrations in terms of the known bulk values, e.g., on the right

$$c_i = c_i'' \exp\left[-zF(V - V'')/RT\right], x \geq d. \tag{18}$$

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which correspond to the double-layers. The thickness of each layer is K^{-1} (for 10^{-3} M ionic strength, 10 nm) and the dielectric constant is ϵ_w (80).

For the special case where $\epsilon_p = \epsilon_w$ and the double-layer is thick, a solution for the potential on the axis of the pore has been provided by Jordan (1982) using an extension of a method introduced by Levitt (1978). For the values used by Jordan to describe gramicidin (given above), this model predicts that the difference in potential between the center of the mouth of the pore and the surface of the unmodified membrane, $V^{\text{mouth}} - V^d$, is 5% of V_m. For comparison, $V^d - V''$ is 8% of V_m. The value of C_m^* required to make $V_d - V''$ in the theory equal to the potential at the mouth of the pore is thus given approximately by $C_m^*/C_m = (5 + 8)/8 = 1.6$.

This value should be taken as an upper limit for three reasons. First, the location at which we require to know the potential is not the center of the pore's mouth, but rather the position of an ion about to enter the pore (see footnote 2). This position is some distance, perhaps 0.1 nm, out into the solution. Jordan calculates that the potential on the axis of the pore relative to the membrane surface falls rapidly with distance into the solution. Second, the value of C_m^* is required to predict only the changes in the potential near the mouth of the pore when the ionic strength is changed from low to high values, not the entire difference between its value and that in the solution at low ionic strength. (The rest of the difference, that present even at say 1 M ionic strength, is treated as part of the actual pore transfer process.) Jordan concludes that "Even 1 M electrolyte cannot substantially affect the potential profile since the electric field is reasonably well confined to the vicinity of the membrane." Third, $V^{\text{mouth}} - V^d$ is probably overestimated because the materials available as part of the pore cannot yield an average dielectric constant of the pore as large as that of bulk water. $V^{\text{mouth}} - V^d$ will decrease with the value assumed for the dielectric constant of the pore, and for the extreme case for which $\epsilon_p = \epsilon_h$, it is exactly zero. If we assume that this variation is linear in $\epsilon_p - \epsilon_h$, and guess that a reasonable value for the dielectric constant is perhaps 20, then C_m^*/C_m falls, even if this is the only reason, to $(1.15 + 8)/8 = 1.14$. Thus, at present there is no compelling theoretical argument that C_m^* for gramicidin should substantially exceed C_m.

[4]Andersen (1983*a*) has argued that the high potential currents at 100 mM are limited by access to the pore. If this process were access to empty pores, then it would also be limiting for all lower concentrations and the current-concentration curve in the absence of double-layer polarization would be linear. The observed nonlinearity, [I(10 mM) × 100 mM]/[I(100 mM) × 10 mM] = 1.5, could then be ascribed to double-layer polarization, which leads to C_m^* near 10 nF/mm^2. However, as discussed elsewhere (Hladky and Haydon, 1984), the limiting process for CsCl at 100 mM is much more likely to be entry of ions into pores that are already occupied. If so, (see Eq. 9, in Urban and Hladky, 1979), the current-concentration curve in the absence of double-layer polarization can be nonlinear. For example, if the rate constants for entry to empty and singly occupied pores, A and D, and for exit from singly and doubly occupied pores, B and E, were independent of potential, D/E were much less than 1, and the rate constant for transfer from left to right, K', were very large, then $I = zFAc\,[1 + Dc/B]/[1 + 2Ac/B]$.

The nonlinearity in this relation between the current at high potentials and the concentration arises because at low concentrations, Ac, $Dc \ll B$, K, E, all ion entries into the pore contribute to the current, while at intermediate concentrations, $B \ll Ac$, $Dc \ll K$, E, some entries lead to exits from the same end that don't contribute. If $A = D$ and $A/B = 100$ M^{-1}, then [I(10 mM) × 100 mM]/[I(100 mM) × 10 mM] = 1.27, while for $A/B = 10$ M^{-1} it is 1.37. The remaining portion of the nonlinearity might then correspond to C_m^* near 5 nF/mm^2. Clearly, further data are required to calculate C_m^*. Andersen (1983*c*) reports a linear current-concentration relation at high potentials for constant, high ionic strength and hence presumably a constant extent of double-layer polarization, but the interpretation of these data depends upon assumptions about the effects of high concentrations of TEACl.

[5]The weak, apparently linear variation of the current at high potentials could result from either a weakly potential dependent limiting step in the actual transport process or from double-layer polarization. If the potential dependence arises entirely from double-layer polarization, then C_m^* calculated from the slope is ~15 nF/mm^2. Andersen (1983*a*) notes that a very weakly potential dependent limiting step would reduce the calculated value to 10 nF/mm^2. Two arguments were presented that the potential dependence does not reduce C_m^* even further: first, that C_m^* can be estimated from the current-concentration curve, and second, that the current-voltage relation is almost flat in the presence of high concentrations of TEACl. The first argument is only as good as the other estimate of C_m^*; the second depends upon the properties of TEACl.

When the potentials are small, the exponentials can be expanded in a power series in V, all but the constant and linear terms discarded, and the results substituted into Eq. 11. The differential equation can be solved simply to yield

$$V = V' + (V^o - V') \exp [Kx], x < 0 \quad (19)$$

and

$$V = V'' + (V^d - V'') \exp [-K(x - d)], x > d \quad (20)$$

where the Debye constant is given by Eq. 4. Within the aqueous phases the displacement vector, **D**, is related to the potential by

$$\mathbf{D} = -\epsilon\epsilon_o(dV/dx). \quad (21)$$

Thus, in the aqueous phase at the left membrane surface, $x = 0$

$$\mathbf{D} = -C_K(V^o - V'), \quad (22)$$

and at the right membrane surface

$$\mathbf{D} = -C_K(V'' - V^d). \quad (23)$$

Within the membrane there are no free charges and thus the displacement vector, **D**, is a constant,

$$\mathbf{D} = -(V^d - V^o)C_m. \quad (24)$$

At the membrane surfaces, the displacement vector undergoes a discontinuity equal to the surface concentration of free charges, i.e.,

$$\mathbf{D}_m - \mathbf{D}_a^o = \sigma^o \quad (25)$$

and

$$\mathbf{D}_a^d - \mathbf{D}_m = \sigma^d. \quad (26)$$

Eqs. 21–26 lead directly by elementary algebra to Eqs. 1 and 2 in the text.

This system is electrically equivalent to a parallel plate capacitor containing three flat layers of dielectric, which in turn is equivalent to three capacitors in series, one containing each of the layers. The inner layer with capacitance C_m is the membrane proper. The outer layers, each with capacitance C_K, correspond to charge-free slabs of water of thickness K^{-1}. The membrane surfaces, which in the theory are isopotentials, are the plates between the capacitors. The charge on each of them corresponds to the sum of the adsorbed charge and the difference between the dielectric polarization in the water and in the membrane. The charge on the outer plates is the net ion accumulation in the diffuse double-layers.

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