LETTER TO THE EDITOR

The Effects of Double-Layer Polarization on the Conductance of Gramicidin Channels. A Reply to O. S. Andersen

Dear Sir:

O. S. Andersen (1983) has considered the currents carried by ions through gramicidin pores and has demonstrated that additions of indifferent electrolytes change the shape of the current-voltage relations at high potentials as expected from the Gouy-Chapman theory for double-layer polarization. Similar changes can be observed at low potentials and these are also consistent with double-layer polarization (Hladky, S. B., unpublished results). Andersen, however, goes on to conclude that this polarization distorts the conductance-concentration relation sufficiently that (p. 144) "the analysis of such data by fitting theoretical conductance expressions to the data . . . is subject to considerable uncertainties."

Three observations are pertinent. First, for pores that are at most singly occupied and in the absence of any indifferent electrolyte, the expected effects of polarization on the conductance-concentration relation disappear in the limit of zero potential. (This follows immediately from Andersen's Eqs. 34 and 39.) Second, the conductance-concentration relation at zero potential can be determined from the conductance-concentration relation at any finite potential and the current-voltage relations. In practice, for gramicidin the change in the ratio $g(0)/g(50 \text{ mV})$ is much smaller than the change in $g(50 \text{ mV})$, and the values of $g(50 \text{ mV})$ can be used directly with only small errors (see p. 366, Hladky, 1984). Third, the available experimental results for potentials below 100 mV strongly suggest that despite important effects above 100 mV the conductance-concentration relations below 50 mV have not been distorted. If such distortion were important, it would affect all permeant ions, although to differing extents, and the effects would be markedly altered by adding indifferent salt. Experimentally, there is no deviation from a simple Langmuir binding curve for sodium from 10 mM to 2 M (Hladky, 1974; Neher et al., 1978). Similarly, for thallium at 1 mM, where the effects are expected to be more pronounced, additions of magnesium sulphate had little effect (Neher et al., 1978). Furthermore, the data reported by Andersen for additions of tetraethylammonium chloride to 10 mM solutions of caesium, potassium, rubidium, or ammonium chloride also show little change in the currents at low potentials. Thus, at low concentrations, double-layer polarization must be considered in the analysis of all data obtained at high potentials and in any interpretation of the shape of current-voltage relations. However, the changes in the current that double-layer polarization produces at low potentials are sufficiently small that they do not cast serious doubt on the interpretation of conductance-concentration relations by Neher et al. (1978) and by Hladky and Haydon (1984).

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On the Effects of Interfacial Polarization. A Reply to S. B. Hladky

Dear Sir:

S. B. Hladky suggests that my data and calculations on interfacial polarization effects (Andersen, 1983) cast no serious doubt on the interpretation of conductance-activity relations measured at low potentials. The purpose of this reply is to show how Hladky's arguments support my conclusion: that there are considerable uncertainties involved in such interpretations. Hladky lists three points in support of his argument.

The first point was made on pages 142–143 in my article (Andersen 1983); see Eqs. 32–36 and Eqs. 37–41, and, in particular, Eqs. 32, 34, and 36, and Eqs. 37, 39, and 41.

In regard to the second point, the concentration-dependent changes in the conductance measured at any fixed potential will be larger than the changes in the ratio of the small-signal (zero-voltage) conductance, $g(0)$, and the measured conductance. It is likewise reasonable to assume that single-channel conductances measured at 50 mV, $g(50)$, should be valid estimators for $g(0)$. One may, however, run into difficulties at low salt concentrations if the data are used to distinguish among different models of ion permeation: With the parameters used to generate Fig. 7 in Andersen (1983), $g(50)$ is ~6% too large in 0.01 M monovalent salt, whereas $g(100)$ is ~17% greater than its value in the absence of interfacial polarization. (The corresponding distortions in 0.001 M monovalent salt are 16 and 51%, respectively. The impact of these small conductance changes must be evaluated in light of the potentially critical importance of accurate conductance data at low salt concentrations, to distinguish among qualitatively different kinetic descriptions of ion movement
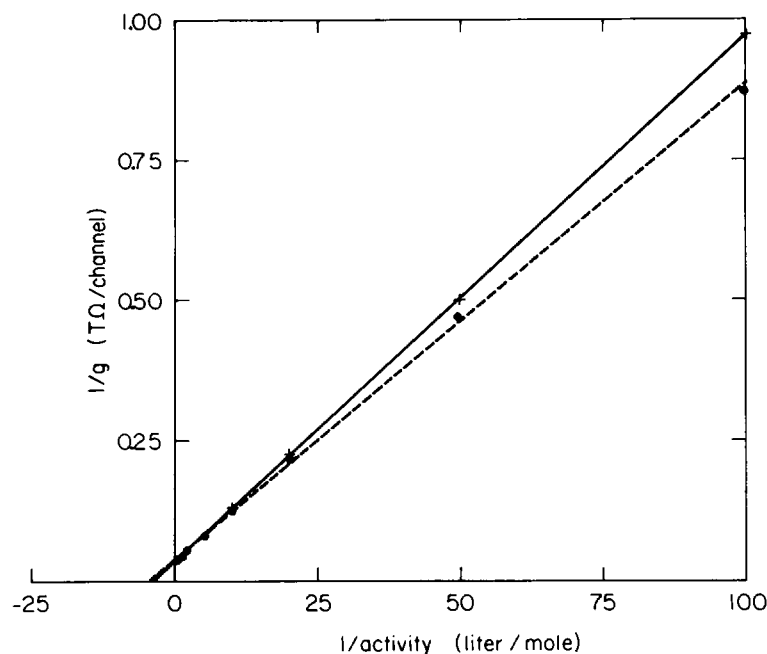
FIGURE 1 Simulated conductance vs. activity data for a channel that can be occupied by at most one ion. The simulations are based on the model in Andersen (1983). The single-site dissociation constant, K, is 0.4 M; the dissociation rate constant, k_{-1}, is 2×10^7 s^{-1} and the translocation rate constant, l, is 10^8 s^{-1}; the fractional voltage-dependencies of the association, dissociation and translocation rate constants are 0.1, 0.1, and 0.3, respectively; the specific capacitance of the channel and surrounding membrane is 1 μF cm^{-2}. The activities for half-maximal conductances $K_1(V)$ listed in the text were estimated from fitting the classical single–ion-occupancy model to the simulated data, by minimizing the sum of the relative errors squared. The figure depicts Lineweaver-Burke plots of conductance data between 0.01 and 2.0 M monovalent salt. X and ——: simulation without interfacial polarization; ● and -----: simulation with interfacial polarization, note that the points fall right around the straight line, suggestive of simple Langmuirian behavior. 100 mV, 25°C.

through a channel (e.g., Hagglund et al., 1979), and the inability to correct low-potential conductance data for interfacial polarization effects in the absence of a preexisting kinetic description of ion movement through the channel (Andersen, 1983).

In regard to the third point raised by Hladky, note that the expression of interfacial polarization depends upon the detailed kinetics of ion movement through the channel (Andersen, 1983). There is no reason to expect the single-channel currents for all permeant ions to be similarly affected, and the presence or absence of interfacial polarization effects for one ion does not eliminate the need to analyze the situation for other ions. Note further that the experimental arguments advanced against a significant influence of interfacial polarization effects are indecisive. First, inspection of Fig. 4 of Neher et al. (1978) shows that the conductances in the presence of MgSO$_4$ are at least 5–15% less than the conductances in thallium acetate alone. There are several reasons why the addition of MgSO$_4$ could decrease the single-channel conductances, and these results are not strong arguments for the existence of interfacial polarization effects. The conductance changes are, however, of the correct magnitude for such effects! Second, conductance vs. activity relations for singly occupied channels may appear Langmuirian even though they are affected by interfacial polarization (see Fig. 1). The scatter in the simulated points around the interrupted line is, if anything, less than the scatter in Fig. 7 in Hladky (1974). The activity for half-maximal conductance estimated from the simulation with interfacial polarization is 0.230 M at 100 mV (or 10% less than the true value of 0.254 M); the corresponding values at 50 mV are 0.210 and 0.217 M, respectively. This describes a best

case situation, as it is assumed that the data will be analyzed according to the classical single–ion-occupancy model. A real problem would have arisen if the small deviations from ideal behavior were interpreted to reflect multiple-ion occupancy or some other characteristic of the kinetics of ion permeation through the channel.

The point, as I see it, is that while conductance-activity data in the low-potential region should be effectively devoid of interfacial polarization effects, then we do not always know what is meant by low—even 50 mV may be a high potential. To the extent that the full current-voltage characteristics are used in the data analysis (e.g., Hladky et al., 1978; Eisenman and Sandblom, 1983), the interfacial polarization will become more pronounced the larger the potential range. This will limit the detailed analysis of the energy barriers for ion movement through the channel, a question not considered by Hladky.

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