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COMPARISON OF THE GRAMICIDIN A POTASSIUM/SODIUM PERMEABILITY AND SINGLE CHANNEL CONDUCTANCE RATIO

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If the ion concentration is low enough that most channels are unoccupied, then the 'independence relations' should be satisfied and the permeability ratio should equal the conductance ratio. It has been previously reported that for the gramicidin A channel these ratios for Na^+ and K^+ were not equal at concentrations as low as 10 mM. However, these ratios were not measured at the same applied potential, as is required by the theory. Instead, the conductance ratio was measured at 100 mV and corrected using calculated current-voltage relations. In this report the comparison between permeability and conductance ratios is reexamined using data obtained at the correct potential. There is no significant difference in the ratios at 10 mM when they are measured at the same voltage. This implies that most channels are not occupied by sodium or potassium ions at 10 mM.

Although the channel formed by gramicidin is probably the simplest and best characterized ion channel, there is still no general agreement on the details of the transport mechanism. In particular, estimates for the dissociation constant for binding of the first potassium ion in the channel range from 1.2 to 365 mM [1]. The strongest evidence for the high-affinity values comes from a comparison of the conductance (G_K/G_N) and permeability (P_K/P_N) ratios of Na^+ and K^+ at low concentrations [2,3]. The permeability ratio is defined by the expression for the bi-ionic potential (Ψ):

$$\Psi = (RT/zF) \ln(P_K/P_N)$$

where Ψ is the measured potential when equal concentrations of Na^+ and K^+ are on opposite sides of the membrane. The channel conductance is defined as the single-channel current divided by applied voltage when there are identical solutions of either K^+ or Na^+ on both sides of the membrane. If the channel obeys the 'independence principal' then the permeability ratio should equal the ratio of the conductance measured at the same concentration at an applied potential equal to the bi-ionic potential [4,5].

For 10 mM Na^+ and K^+ Urban et al. [2] reported a bi-ionic potential of 27.7 mV (corresponding to a permeability ratio of 2.95 at t = 24°C) and a conductance ratio of 2.40 at 100 mV. Since model calculations indicated that the conductance was relatively independent of voltage for voltages less than 100 mV, it was concluded that the conductance ratio was about 2.4 at 27 mV. This conclusion is supported by the observation of Neher et al. [6] of a conductance ratio at 50 mV of 2.56 ± 0.09. This difference in the two ratios suggested that gramicidin did not satisfy the independence condition at 10 mM. The necessity of explaining this observation was one of the main reasons for favoring a high-affinity model with a large fraction of the channels containing either an Na^+ or K^+ at concentrations as low as 10 mM [3].

The purpose of these experiments is to measure directly the conductance ratio at 27 mV and de-

termine whether it differs from the permeability ratio. These measurements are difficult because the combination of low concentration (10 mM) and low applied potential (27 mV) means that the single channel currents are small.

The bilayer membranes (1% glycerol monolein in hexadecane) were formed on the end of a 0.3 mm internal diameter teflon tube as described previously [1]. The area of the thin portion of the membrane was about 0.02 mm^2. The gramicidin (ICN) was usually added to the aqueous solution. The single channel currents were measured using an amplifier similar to that described by Hamill et al. [7]. The Ag-AgCl electrodes had an offset voltage of at most 2 mV. Experiments were discarded if the offset voltage changed by more than 1 mV during the experiment. As an additional check that the correct offset voltage was used, each set of measurements was made with positive and negative applied voltages. If the two measurements differed by more than 3% the experiments were discarded. The current was filtered through a sixth order low pass Bessel filter with a 3 Hz corner frequency. Only channels at least one second long were used (Fig. 1, inset). The single-channel currents were recorded on a strip-chart recorder and measured by hand. The bi-ionic potential measurements were made using a similar apparatus except that larger membranes (0.3 mm^2) were used. The front and back chamber was filled with one electrolyte solution (either NaCl or KCl) and a drop of the membrane forming solution was placed on the end of the teflon tube. Then the front chamber was filled with the other electrolyte and the membrane suddenly thinned. This allowed the measurement of the bi-ionic potential to be made within one second of the thinning, and before any unstirred layers could develop. The gramicidin concentration (and, therefore the ion flux) was always low enough that there was no significant change in the bi-ionic potential for at least 1 min after thinning. All experiments were at room temperature (approx. 25°C).

The bi-ionic potential with 10 mM NaCl and KCl on opposite sides of the membrane was 27.2 $\pm$ 0.3 (S.E.) mV ($N = 4$). This is similar to the value of 27.7 mV obtained by Urban et al. [2].

A typical current recording is shown in Fig. 1 (inset) for 10 mM NaCl and KCl at an applied

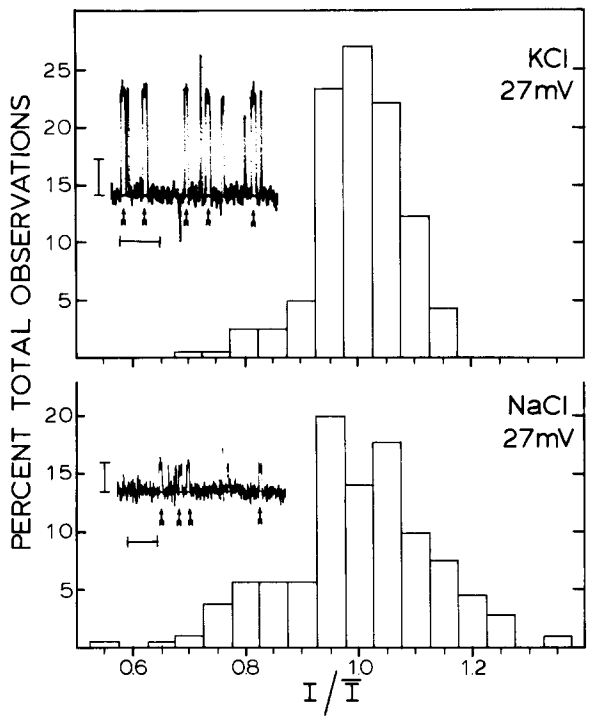

Fig. 1. Frequency histogram of the normalized single channel current ($I/\bar{I}$) for 10 mM KCl ($N = 163$) and NaCl ($N = 214$) at an applied voltage of 27 mV. The insets show representative recordings of the single-channel current. The calibration bars indicate 0.025 pA and 10 s. The arrows indicate the channels that were open 1 s or longer and from which the measurements were taken.

potential of 27 mV. Fig. 1 shows the normalized histogram of the single channel measurements for Na^+ and K^+ at 10 mM and 27 mV. The two ions have essentially the same frequency distribution except that the Na^+ peak is not as sharp as that for K^+, presumably because of the lower signal to noise ratio for Na^+. Some of the variation observed in Fig. 1 probably results from the fact that commercial gramicidin A contains small amounts of gramicidin B and C [8]. However, since all three types have the same K^+/Na^+ conductance ratio [9] and both ions have the same frequency distribution (Fig. 1), the ratio of the average values should represent the true ratio of the single-channel conductances. The averages values are summarized in Table I. The conductance ratio at 10 mM and 100 mV is 2.45, similar to the value of 2.40 obtained by Urban et al. [2]. The conductance ratio at 27 mV is 2.91. This voltage dependence of

TABLE I

SINGLE CHANNEL CURRENT AND CONDUCTANCE AT 10 mM

	NaCl		KCl	
	27 mV	100 mV	27 mV	100 mV
Current (pA)	0.0235	0.0704	0.0682	0.172
Conductance (pS)	0.869	0.704	2.53	1.72

the conductance ratio is in quantitative agreement with the recent many channel measurements of Eisenman et al. [10].

The bi-ionic potential for 10 mM K^+ and Na^+ of 27.2 mV corresponds to a permeability ratio of 2.89. This is not significantly different from the conductance ratio (2.91) measured at the same applied potential. Thus, gramicidin does obey the 'independence principle' at 10 mM. The previous conclusion that the two ratios were not equal resulted from the use of the conductance ratio measured at 100 mV (2.45).

This identity of the permeability and conductance ratio means that the flux of, for example, Na^+ in the bi-ionic experiment is not affected by the presence of K^+ on the other side of the membrane. This would not be the case if a significant number of channels were occupied by K^+ at 10 mM. Thus, this agreement with the independence principal places a lower limit on the dissociation constant of the gramicidin channel for Na^+ or K^+. Although a quantitative value for this lower limit requires an analysis of a specific kinetic model, one can make the following rough estimate. A dissociation constant of 10 mM would mean that about 50% of the channels are occupied by K^+ (in the equilibrium case) and this should be detectable as a deviation from independence. Thus, one can conclude from these results that the dissociation constant for K^+ and Na^+ should be greater than 10 mM.

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