PROTONS DECREASE THE SINGLE CHANNEL CONDUCTANCE OF THE SARCOPLASMIC RETICULUM K^+ CHANNEL IN NEUTRAL AND NEGATIVELY CHARGED BILAYERS

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ABSTRACT The conductance of rabbit sarcoplasmic reticulum K^+ channels incorporated into artificial bilayers of varying lipid composition was measured at different K^+ and proton concentrations. Protons competitively inhibit the K^+ conductance with a K_i of 0.5 μM. In negatively charged membranes, the conductance is well described by Gouy-Chapman-Stern theory modified to include the inhibitory effect of protons of the conductance and assuming that the channel mouth is isolated by 5–10 Å from bilayer surface.

INTRODUCTION

Bell and Miller (1984) have reported that the single channel conductance of the rabbit sarcoplasmic reticulum (SR) K^+ channel varies with the surface potential of the surrounding membrane. The conductance of the channel changes in a manner consistent with an electrostatic effect of the phospholipid surface charge on the K^+ concentration near the channel mouth (for a review of surface potential theory see McLaughlin, 1977). By fusing SR vesicles containing the channel into artificial bilayers of defined phospholipid compositions, they showed that at a given K^+ concentration the channel conductance is larger in negatively charged membranes and smaller in positively charged ones in comparison with conductances obtained in net neutral membranes. However, in addition to changing the K^+ concentration, the surface potential will also influence the proton concentration at the membrane surface. For example, a surface pH as low as 4.5 can be calculated in some of their experiments designed to measure channel conductance. The possible effect of such a high proton concentration on the channel conductance has not yet been examined in detail.

Labarca (1980) showed that protons can inhibit the single channel conductance of this channel with half-maximal inhibition at pH 5.5 (at 200 mM K^+). This proton concentration is clearly lower than that mentioned above. A possible consequence of this observation is that the conductance in the negatively charged membranes will be determined not only by the effect of the charged phospholipids on the K^+ concentration but also by the effect on the H^+ concentration. The effects of K^+ and H^+ are antagonistic: an increased K^+ concentration with respect to the bulk value will tend to increase channel conductance while an increased H^+ concentration will tend to decrease the conductance. If this effect of the protons is ignored, the magnitude of the surface potential effect calculated from the measured conductances will be underestimated.

Therefore, to analyze properly the effect of surface charge on the channel conductance it is necessary to examine the interactions of protons with the channel and to modify the theory used to interpret the data. This paper describes experiments which demonstrate that protons act as competitive inhibitors of the K^+ conductance with an inhibition constant, (K_i) of 0.5 μM. The single channel conductances in membranes containing 15 and 70% negative charge (phosphatidylserine) measured at pH 8.5 were compared with those measured at pH 7.2. The conductances were larger at the higher pH. When the data are analyzed by Gouy-Chapman-Stern theory modified to include the true K_M (i.e., K_M in the limit of zero protons) for K^+ as well as the K_i for protons, it is shown that the theory fits the data better than when the proton effect is ignored. The main conclusion of this work is not different from that of Bell and Miller (1984): The channel is affected by surface charge in a manner consistent with the pore entryway being 5 to 10 Å from the membrane surface. However, the effect of pH on the single channel conduc-

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tance must be considered to interpret the magnitude of the conductances in negatively charged membranes.

METHODS

Phosphatidylserine (PS), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) were purchased from Avanti Biochemicals, Inc. (Birmingham, AL) and not further purified. Aqueous solutions contained the appropriate concentration of K^+ gluconate, and either 5 mM MOPS (morpholinopropanesulfonic acid) (in solutions adjusted to pH 7.2) or 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (in solutions adjusted to pH 8.5). Single channel conductances were measured by fusing SR vesicles containing the channel (prepared from rabbit back and leg muscle as in Garcia and Miller, 1984) to artificial bilayers of the desired phospholipid composition. Negatively charged bilayers were made from 20 mg/ml solutions of either 70% PS/30% PE or 15% PS/85% PE dissolved in pentane. Neutral bilayers were made from solutions containing 70% PE/30% PC. Membranes were made by folding together two lipid monolayers by the technique of Montal and Mueller (1972). Methods for obtaining fusion are described in detail in Bell and Miller (1984). Single channel fluctuations were recorded at holding potentials between +60 and −60 mV.

Theoretical predictions of the effect of surface potential on the single-channel conductance were calculated from the measured charge density. These measurements are described in detail in Bell and Miller (1984). Membranes made from 15% PS/85% PE have a charge density of 0.21 ± 0.02 charges/nm^2, while those containing 70% PS/30% PE have a charge density of 0.9 ± 0.2 charges/nm^2. The charge density was measured at a bulk pH of 7.2 only. Because PS has a titratable carboxyl group (pK = 3), it was considered that the fraction of negative charge in the membrane might therefore be a function of pH and/or K^+ concentration. However, the measured charged density did not vary with K^+ concentration [from 10–100 mM]. Furthermore, assuming that the surface pH was never lower than 4.5, one can calculate from the above pH that <5% of the PS is neutralized under these conditions. Knowing the surface charge density it is possible to calculate the surface potential, ψ_o, the decay of the potential with distance from the charged surface, and thus the electrolyte concentration at various distances from the bilayer using Gouy-Chapman-Stern theory (McLaughlin, 1977). The potential at a given distance x is given by

$$\psi(x) = \frac{2RT}{F} \ln \frac{1 + \alpha \exp(-\kappa X)}{1 + \alpha \exp(-\kappa X)}, \quad (1)$$

where κ, the reciprocal Debye length is

$$\kappa = \left[\frac{2F^2 C(\infty)}{\epsilon\epsilon_0 RT}\right]^{1/2} \quad (2)$$

and

$$\alpha = \frac{\exp(F\psi_o/2RT) - 1}{\exp(F\psi_o/2RT) + 1}. \quad (3)$$

F, R, and T have their usual meanings, ϵ and ϵ_0 are the dielectric constant and permittivity of free space, respectively, $C(\infty)$, the bulk electrolyte concentration, and ψ_o, the potential at the charged surface. The surface potential, ψ_o, used in Eq. 3 was obtained iteratively from the measured charge density and the bulk electrolyte concentration according to

$$\sinh \frac{(F\psi_o)}{(2RT)} = \frac{\sigma}{[1 + K_a C(\infty) \exp(-F\psi_o/RT)]\,[8\epsilon\epsilon_0 RTC(\infty)]^{1/2}}, \quad (4)$$

where σ is the charge density in charges/Å^2, and K_a is the association constant of K^+ for PS, here taken to be 0.15 M^{-1} (Eisenberg et al., 1979). This equation thus accounts for the effects of screening as well as specific binding by K^+ on the magnitude of the surface potential. From these four equations we can determine the electrostatic potential at any distance from the membrane. Furthermore, we can calculate the concentration of a given cation (for our purposes, these will be protons and K^+) at any distance x from the membrane by noting that

$$C^+(x) = C^+(\infty) \exp[-F\psi(x)/RT] \quad (5)$$

where C^+ is the concentration of a given cation in the bulk solution, and $C^+(x)$ the concentration at distance x.

RESULTS AND DISCUSSION

Fig. 1 shows a Lineweaver-Burke plot of single channel conductance in PE/PC membranes as a function of K^+ concentration at pH 5.5, 6.0, and 7.2. From this plot it appears that protons competitively inhibit the K^+ conductance with a K_i of 0.5 μM. The true K_M for K^+ obtained from these data is 31 mM.

Fig. 2 *a* plots the channel conductance as a function of K^+ concentration in membranes containing 15% PS/85% PE at pH 7.2 and 8.5. Fig. 2 *b* are data obtained from 70% PS/30% PE bilayers. The dashed line in both graphs indicates the best fit of the channel conductances observed in neutral (PE/PC) membranes from the measured γ_{max} of 220 pS and K_M of 31 mM (data not shown). It is clear that regardless of pH the conductances in the PS membranes are greater than in the neutral bilayers particularly at the lower K^+ concentrations. As the K^+ concentration is raised the conductance in the charged membranes approaches the γ_{max} observed in PE/PC bilayers (i.e., 220 pS). At both concentrations of PS the conductances are larger at pH 8.5

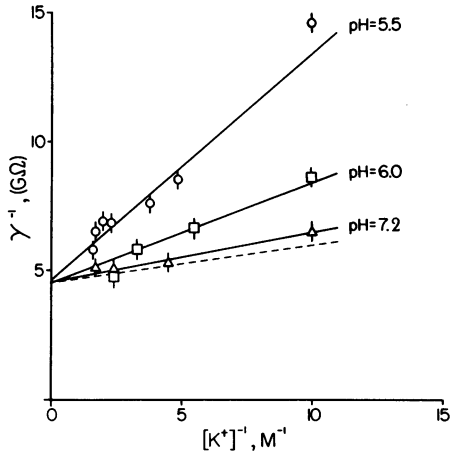

FIGURE 1 Competitive block of K^+ conductance by H^+. Channels were incorporated into PE/PC bilayers and the conductance measured at several voltages. Data were obtained at three H^+ concentrations and are plotted in double-reciprocal form against K^+ concentration. The K_i for H^+ was determined by noting that $K_i = (K'_{Mapp}H^+ - K_{Mapp}H^+)/(K_{Mapp} - K'_{Mapp})$, where K_{Mapp} is the K_M apparent at proton concentration H^+, and K'_{Mapp} the K_M apparent at $H^{+\prime}$. The K_i obtained in this manner is 0.5 μM. The true K_M for K^+ was then calculated from $K_M = (K_{Mapp})/[1 + (H^+/K_i)]$ and is equal to 31 mM. The dashed line represents the expected slope at K_M = 31 mM and γ_{max} = 220 pS.

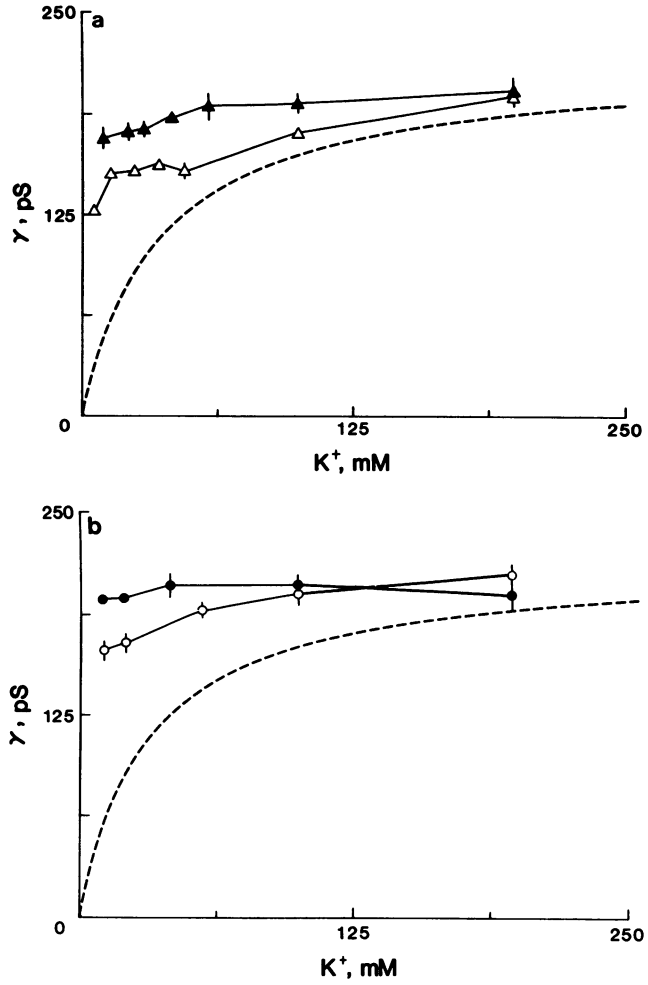

FIGURE 2 Effect of pH on single channel conductance in negatively charged membranes. Channel conductance was measured at the indicated K^+ concentrations in bilayers composed of two different charge densities. Data represent mean and standard error of at least 10 measurements in 3 different membranes. The dashed line in *a* and *b* represents conductances obtained in neutral membranes. (*a*) Conductances measured in bilayers made from 15% PS/85% PE. Closed triangles are data obtained at pH 8.5, open triangles at pH 7.2. (*b*) Conductances measured in bilayers made from 70% PS/30% PE. Closed circles are data obtained at pH 8.5, open circles at pH 7.2.

than at pH 7.2 again, particularly at the lower K^+ concentrations.

The evidence that at a given pH the differences in the conductance vs. K^+ concentration profiles between neutral and negatively charged bilayers are due to surface potential has been presented and discussed elsewhere (Bell and Miller, 1984). In essence, the conductance at a given bulk K^+ concentration in charged membranes is higher than in the neutral ones because of the increased K^+ concentration (due to the negative surface potential) in the vicinity of the channel. This explanation invokes only an electrostatic effect of the charged phospholipid on the ion concentrations near the membrane surface and does not involve any alteration of inherent channel properties, i.e., the K_M for K^+ and V_{max} remain unchanged. The effect of pH on the conductance in the PS membranes reported here is consistent with this explanation. The proton concentration as well as the K^+ concentration at the surface of the membrane will be higher than that in the bulk solution. Because protons inhibit the K^+ conductance one would expect that at the same K^+ concentration the conductance will be greater at a higher pH. The effect is more pronounced at lower K^+ concentrations because protons are a competitive inhibitor of K^+ conductance. Therefore, the conductance increase observed when the pH is raised from 7.2 to 8.5 can also be explained by a purely electrostatic effect, in this case, on the proton concentration near the channel entryway. By including the effect of surface potential on the proton as well as the K^+ concentration at the membrane surface, we can determine if the conductances observed at the different pH values are indeed in agreement with those predicted from an electrostatic mechanism.

In this treatment, the conductance at a given bulk K^+ and H^+ concentration is given by

$$\gamma = \frac{\gamma_{max}}{1 + K_{mapp}/K^+ \exp(-F\psi/RT)} \quad (6)$$

where γ is the channel conductance; γ_{max}, the maximum single channel conductance; K^+, the bulk K^+ concentration; ψ, the potential at the entrance of the conductance pathway. K_{Mapp} is the apparent K_M for K^+ and is given by

$$K_{Mapp} = K_M\{1 + [H^+ \exp(-F\psi/RT)/K_i]\} \quad (7)$$

where K_M is the true K_M for K^+; H^+, the bulk proton concentration; K_i, the inhibition constant for protons. Thus the measured conductance can be used to calculate the potential sensed at the pore

$$\exp(-F\psi/RT) = \frac{K_M}{\frac{\gamma max K^+}{\gamma} - K^+ - \frac{K_M H^+}{K_i}}. \quad (8)$$

TABLE I
PREDICTED AND OBSERVED EFFECTS OF pH ON SINGLE CHANNEL CONDUCTANCE IN PS/PE MEMBRANES

Lipid	K^+	$\gamma(7.2)/\gamma(8.5)$	
		Observed	Predicted
	mM		
	14	0.81–0.85	0.93
15% PS	100	0.84–0.95	0.88
	32	0.80–0.86	0.91
70% PS	14	0.80–0.86	0.77
	24	0.83–0.92	0.86

Predicted conductance ratios at pH 7.2 and pH 8.5 were calculated by noting that a given pH: $\gamma = \gamma_{max}\, K^+/K^+ + K_M/\exp(-F\psi/RT) + K_M H^+/K_i$. K^+ is the bulk K^+ concentration; H^+, the bulk H^+ concentration; K_M and K_i have the values mentioned in the text, namely, 0.031 and 5×10^{-7} M, respectively. By solving for $\exp(-F\psi/RT)$, the expected conductance at a different H^+ concentration was then determined. The range of observed ratios includes the standard errors of the measured values.

Because the potential is determined by the bulk ionic strength, to which protons contribute a negligible amount, this potential can be used to predict the channel conductance at identical K^+ concentrations and different pH values. Table I shows that the conductance changes observed in the PS membranes at pH 7.2 and 8.5 are within 10% of the expected shifts predicted from a mechanism in which only the concentration of the competitive inhibitor H^+ is altered. This comparison provides additional evidence that the effects of the changing pH on the measured conductances are primarily electrostatic.

From Fig. 2 *a*, *b* it is clear that the channel conductance is increasing as the bulk K^+ concentration is raised (particularly at pH 7.2). The observed channel conductances do not fit the profile of a channel feeling the entire potential at the surface of the membrane, i.e., at the plane of the lipid head groups. The equations predict that if the channel were sensing the potential immediately adjacent to the surface the conductance would change very little as the bulk K^+ concentration is varied. They also predict that this constant conductance would be very close to γ_{max}. However, the measured conductances clearly increase over the range of 10–200 mM K^+. As discussed in Bell and Miller (1984) a possible explanation for this observation is that the channel mouth is in some way isolated from the membrane surface. By calculating the potential and hence the K^+ and H^+

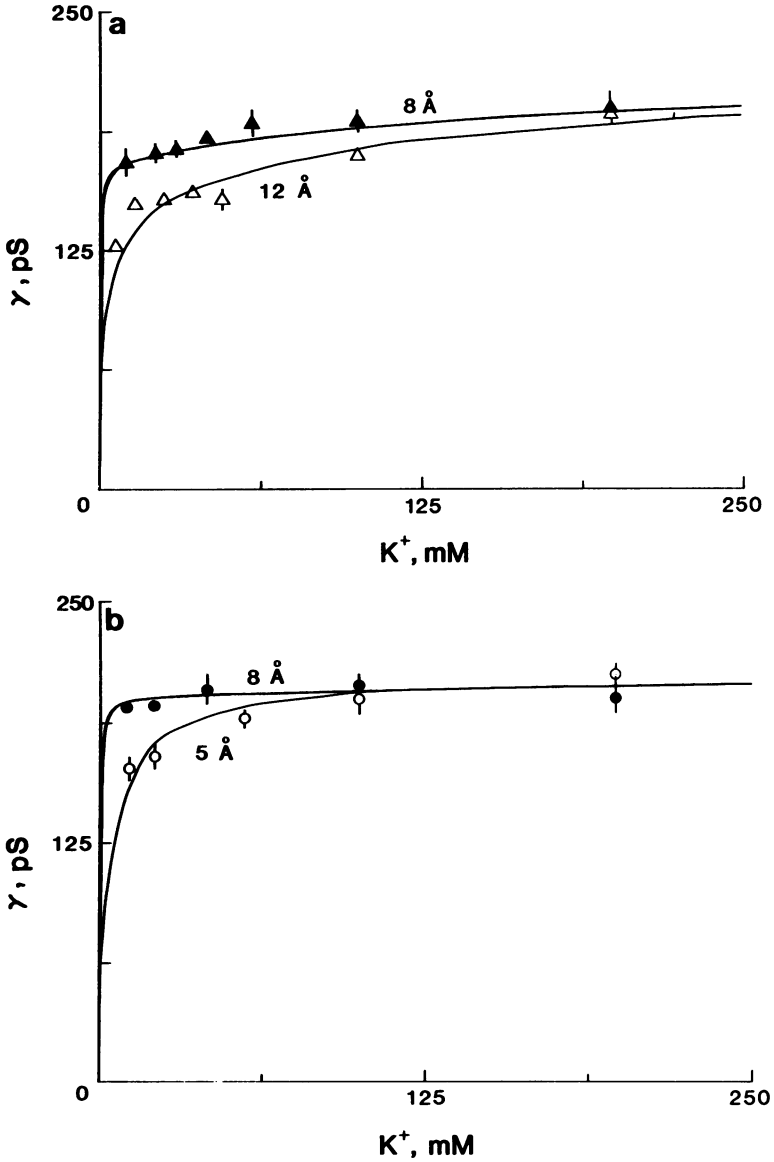

FIGURE 3 Predicted conductance vs. K_i^+ concentration profiles. Curves calculated from Eqs. 1–7 represent the expected conductance of a channel protruding the indicated distance, in Angstroms, from the bilayer. (*a*) Data (from Fig. 1 *a*) are conductances in membranes containing 15% PS/85% PE. Closed triangles are data taken at pH 8.5, open triangles at pH 7.2. (*b*) Data (from Fig. 1 *b*) are conductances in membranes containing 70% PS/30% PE. Closed circles are data taken at pH 8.5, open circles at pH 7.2.

concentrations at various distances from the membrane (as a function of the bulk concentrations) from Eqs. 1–7 we can predict the conductance of the channel assuming a given distance between the channel mouth and the membrane surface. This calculation is similar to that made by Bell and Miller (1984) except that here we include the inhibitory effect of protons. Fig. 3 *a*, *b* show the best fits obtained at the different surface potentials and pHs. The data are well fit by assuming an average distance of approximately 5–10 Å. The fit, particularly at low K^+ concentrations, is clearly better than that obtained without considering the effect of protons on the conductance.

In summary, it has been shown that protons are competitive inhibitors of the K^+ conductance in the SR K^+ channel. Furthermore, it is necessary to consider this effect of protons in the theoretical predictions in order to describe best the effect of surface charge on the single channel conductance. Although the conclusion is not different from that drawn from Bell and Miller (1984), these results explain why the data did not fit their predictions well at low K^+ concentrations.

These data suggest the importance of considering surface potential effects when using lipids bearing a net charge (such as PS, diphosphatidylglycerol, phosphatidylglycerol, phosphatidic acid, or soybean asolectin) in channel reconstitution experiments. Both the pH and the cation and anion concentrations at the membrane surface will be different from those in the bulk solution. The magnitude of the differences will, of course, depend on the charge density of the membrane and the bulk ionic strength. In experiments using asymmetric lipid or solution conditions (as in Labarca et al., 1984; or in patch-clamp experiments where the membrane composition is usually unknown), it is particularly important to know whether the channel under study is sensitive to surface potential, because both the potential across the membrane and ion concentrations near the membrane will be affected. Under these conditions, the explanation of a current-voltage curve or conductance vs. concentration curve will be different for a channel affected by surface potential from one that is not. This concern is particularly applicable to the interpretation of single channel rectifying current voltage curves measured by patch clamp (Fukushima, 1981). It is possible that such rectification may not be due to an inherent property of the channel protein but to the well-documented phenomenon of asymmetric bilayer composition in biological membranes (White, 1973). To date, many ionophores and some channels, including the Ca^{2+}-activated K^+ channel and squid axon Na^+ channel have been shown to have some sensitivity to surface charge (Muller and Finkelstein, 1972; Begenisich, 1975; Hille et al., 1975; Apell et al., 1977; Latorre et al., 1985). Therefore, surface potential effects may often be pertinent to the interpretation of gating and conduction characteristics obtained from ion channels incorporated into artificial bilayers or studied in biological membranes.

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