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Increased Activity of Large Conductance Ca^{2+}-Activated K^+ Channels in Negatively-Charged Lipid Membranes

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The effects of membrane surface charge originated from lipid head groups on ion channels were tested by analyzing the activity of single large conductance Ca^{2+}-activated K^+ (maxi K) channel from rat skeletal muscle. The conductances and open-state probability (P_o) of single maxi K channels were compared in three types of planar lipid bilayers formed from a neutral phosphatidylethanolamine (PE) or two negatively-charged phospholipids, phosphatidylserine (PS) and phosphatidylinositol (PI). Under symmetrical KCl concentrations (3~1,000 mM), single channel conductances of maxi K channels in charged membranes were 1.1~1.7 times larger than those in PE membranes, and the differences were more pronounced at the lower ionic strength. The average slope conductances at 100 mM KCl were 251±9.9, 360±8.7 and 356±12.4 (mean±SEM) pS in PE, PS and PI membranes respectively. The potentials at which P_o was 1/2, appeared to have shifted left by 40 mV along voltage axis in the membranes formed with PS or PI. Such shift was consistently seen at pCa 5, 4.5, 4 and 3.5. Estimation of the effect of surface charge from these data indicated that maxi K channels sensed the surface potentials at a distance of 8~9 Å from the membrane surface. In addition, similar insulation distance (7~9 Å) of channel mouth from the bilayer surface charge was predicted by a 3-barrier-2-site model of energy profile for the permeation of K^+ ions. In conclusion, despite the differences in structure and fluidity of phospholipids in bilayers, the activities of maxi K channels in two charged membranes composed of PS or PI were strikingly similar and larger than those in bilayers of PE. These results suggest that the enhancement of conductance and P_o of maxi channels is mostly due to negative charges in the phospholipid head groups.

Key Words: Large conductance Ca^{2+}-activated K^+ channel, Surface charge, Phosphatidylethanolamine, Phosphatidylserine, Phosphatidylinositol, Planar lipid bilayer

INTRODUCTION

The electrostatic potentials produced by fixed charges at the surface of a plasma membrane could affect the behavior of charged molecules in the aqueous solution, and these effects of surface charges can be quantitatively estimated (McLaughlin, 1977; Lattore et al, 1992). For ion channels, the surface potentials from the charges on the channel protein and/or phospholipid head groups can change the conductance, gating and sensitivity to blockers (Green & Anderson, 1991; Lattore et al, 1992). But, only few reports have been focused on the effect of bilayer surface charge on ion channel activity.

Bell & Miller (1984) have shown that single channel conductances of sarcoplasmic reticulum K^+ (SR-K) channels recorded in planar lipid bilayers were higher in negatively-charged bilayers and lower in positively-charged bilayers than those in neutral bilayers. Such effect was more prominent at the lower ionic strength, suggesting the increase or decrease of K^+ ions at the channel mouth by electrostatic interactions. Similarly Moczydlowski et al (1985) demonstrated that the conductance and gating of the large conductance Ca^{2+}-activated K^+ (maxi K) chan-

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nel were increased in the bilayers formed with negatively-charged phospholipid. In contrast to these K^+ channels, the conductances of voltage-dependent Na^+ channels were insensitive to the membrane surface charge under similar experimental conditions (Green, Weiss & Anderson, 1987; Worley et al, 1992). In addition, the conductances of t-tubule Ca^{2+} channels were also insensitive to the surface charge of the phospholipid bilayer (Coronado & Affolter, 1986).

The reason for different responses of ion channels to the surface charges of the phospholipid bilayer is not known. It is not clear why K^+ channels were sensitive but batrachotoxin-inactivated Na^+ and t-tubule Ca^{2+} channels were not. It could be due either to the intrinsic property of ion channel itself, or to the difference in the properties of the bilayer membranes. For lipid bilayers, the properties of membrane other than charges in the head group, such as acyl chain length, number of double bond and head group structure could influence the activity of an ion channel (Chang et al, 1995; Chen & Gross, 1995; for review see Carruther & Melchior, 1986). So far all the known reports on the effect of negative surface charge on K^+ channels were based on the results studied in bilayers formed from phosphatidylserine (Bell & Miller, 1984; Moczydlowski et al, 1985; Green et al, 1987; Worley et al, 1992). Therefore, the data on the behavior of any ion channel in a negatively-charged bilayers composed of other phospholipids will further help us delineate the effect of surface charge from that of other lipid properties.

The aim of this study is to examine whether the membrane surface charge originated from lipid head groups is the major determinant in increasing the activity of maxi K channels as suggested by Moczydlowski et al (1985). We compared the activities of maxi K channels recorded in bilayers formed with neutral phosphatidylethanoamine with those in bilayers formed with negatively charged phospholipids. The two chosen molecules are phosphatidylserine and phosphatidylinositol, each containing net one negative charge in the head group but different structures in acyl chain and tail. We observed that the conductance and gating activity of maxi K channels were increased in two charged membranes containing phosphatidylserine or phosphatidylinositol with a striking similarity. A preliminary result of this work was previously communicated (Park & Ryu, 1994).

METHODS

Membrane preparation

Membrane vesicles containing large conductance Ca^{2+}-activated K^+ (maxi K) channels were prepared from the rat skeletal muscle by using a sucrose density gradient centrifugation as described previously (Guo et al, 1987; Park et al, 1994). Active microsomes were also obtained by the method of Toro et al (1990) developed for the microsomal preparation from the rat uterine smooth muscle.

Planar lipid bilayers

Three types of lipid bilayers constructed for our studies were: PE membranes formed with 100% palmitoyl-oleoyl-phosphatidylethanoamine (PE), PS membranes formed with 80% palmitoyl-oleoyl-phosphatidylserine (PS) and 20% PE, and PI membranes formed with 80% phosphatidylinositol (PI) and 20% PE. Synthetic PE and PS and natural PI from bovine liver were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Twenty percent of PE in charged membranes was necessary to form stable membranes and to obtain better incorporation of the K^+ channels. Each day fresh lipid solutions were dissolved in decane at a concentration of 25 mg/ml.

Electrophysiological set-up

The *cis*-side of the bilayer where we added the membrane vesicles was kept at ground level while the applied voltage and membrane currents were measured in the *trans* side using a bilayer amplifier (BC525A, Warner Instr, CO. Hamden, CT USA). Both sides of the membrane were connected to the amplifier via a KCl (0.5 M)-agar (3%) bridges. Junction potentials were less than 2 mV and not corrected. Transmembrane potentials were expressed by using the cell convention, $V_{intracellular}$-$V_{extracellular}$. Data were stored in a VTR tape via PCM (VR-10A, Instrutech Co., New York, NY, USA) during experiment. The stored data were replayed at 100~500 Hz cut-off frequency by using an 8-pole Bessel filter and digitized at 2 kHz into a PC for analysis. Current and membrane potentials were also recorded on paper at a thermal pen recorder.

Channel incorporation and recording

Single channel recording was carried out as described previously (Guo et al, 1987; Park et al, 1994). Bilayers were formed by painting with the appropriate lipid solution over a 200 μm hole on a teflon cup. Bilayer formation and its size were determined by measuring capacitance current of the membrane. When a membrane with the capacitance of about 100 pF and the conductance less than 1 pA at 100 mV was formed, an ionic gradient was established by adding 5~30 μl of 3M KCl to *cis* side. Then, we added microsomes to the *cis* compartment and stirred the compartment by a small magnetic bar (1×3 mm). Incorporation of channel was indicated by an abrupt appearance of single channel activity on an oscilloscope screen. To facilitate the incorporation of maxi K channels, we added $CaCl_2$ (100 μM) to *cis* solution and kept the KCl concentration of the compartment higher than that of *trans* compartment by 50~100 mM. The activity of maxi K channel was usually seen in 15~20 min. If no channel was observed within 20 min, a new membrane was formed. The intracellular side of the maxi K channel was determined by its voltage dependence (Moczydlowski & Latorre, 1983). In most cases the *cis* side, to which the vesicles were added, was intracellular.

Recording solution contained 10 mM HEPES and various concentrations of KCl and its pH was adjusted to 7.2 with 2M KOH. When needed, K^+ concentrations were adjusted by either perfusing one side of the chamber or by adding a small volume of 3M KCl. Ca^{2+} concentration was kept at 100 μM by adding concentrated $CaCl_2$ solution for activation of maxi K channels. The relative probability of channel opening was measured at various *cis* Ca^{2+} concentrations (pCa 3.5~5.0). The *cis* Ca^{2+} concentration was adjusted by adding an appropriate amount of EDTA according to Fabiato (1988).

Single channel data stored in VCR tapes were replayed and digitized into a 486 PC. The amplitude of single channel current was measured directly from the computer screen at the voltages of −80 to 80 mV with the aid of pClamp (Ver 6.0). The slope conductance was calculated from the resulting individual current-voltage curves at various KCl concentrations (3~1,000 mM). Open-state probability was determined from the record of longer than 1 min at a given potential with Fetchan of pClamp program at 100 mM KCl and pCa 5.0-3.5. Membranes containing more than one channel were not included for data analysis. All experiments were performed at room temperature.

Measurement of surface charge density

The density of negative surface charges were determined by nonactin method (McLaughlin, 1970; Bell & Miller, 1984). Briefly, nonactin (1 μM) was added to one side of the membrane formed with one of three types of lipids in a buffer containing symmetrical 100 mM K-gluconate and 10 mM HEPES-n-methylglucamine (NMDG) (pH 7.2). The membrane conductances were monitored by applying voltage pulses of 5 sec. The addition of nonactin caused a large increase in membrane conductances. When the nonactin-induced conductances become stable, they were was gradually screened by adding LiCl up to 3M at which there was no further change in membrane conductance. Then, surface potential was calculated by the following equation based on Guoy-Chapman-Stern theory (McLaughlin, 1977; Bell & Miller, 1984).

$$\psi_o = -(RT/F)\ln(G^*/G_o) \quad \text{------} \quad (1)$$

where ψ_o is surface potential (mV) and G^* and G_o are membrane conductances before and after adding LiCl in siemens (S), respectively. R, T and F are gas constant (8.3144 $JK^{-1}mol^{-1}$), absolute temperature (298 K) and Faraday constant (9.648×10^4 $Cmol^{-1}$), respectively.

Equation (1) can be related to surface charge density by the following equation.

$$\delta = (1+K_aK_b \exp(-F\psi_o/RT))(8\varepsilon\varepsilon_oRT/K_b)^{1/2}\sinh(F\psi_o/2RT) \quad \text{----------} \quad (2)$$

where K_b is the bulk K^+ concentration; ε (78.54) and ε_o (8.854×10^{-12} $CV^{-1}m^{-1}$) are the dielectric constant of water and the permeability of free space; K_a, the association constant of K^+ to phospholipid (phosphatidylserne) is taken to be 0.15 M^{-1} (Eisenberg et al, 1979). The measured surface charge densities are summarized in Table 1.

Quantitation of surface charge effects

In this study we first attempted to describe the

Table 1. Surface charge densities of 3 types of bilayers

Bilayer composition	Charges/nm^2 (mean±s.e.m.)	%*
100%PE	0.04±0.01 (n=5)	3
80%PS/20%PE	0.98±0.04 (n=4)	70
80%PI/20%PE	1.07±0.06 (n=3)	76

*Apparent percentage of charged lipid in the bilayer was calculated from the measured charge density, assuming a molecular area of 0.7 nm^2 per phospholipid (Loosely-Millman et al, 1982).

conductances and open state probability of maxi K channels as a function of the concentrations of K^+ or Ca^{2+} accumulated at the vicinity of the channel. Then, we related the concentrations of K^+ or Ca^{2+} as a function of electrostatic potential at distance x from the membrane surface. The calculations based on the surface charge theory (McLaughlin, 1977; Bell & Miller, 1984; Moczydlowski et al, 1985; Latorre et al, 1992).

The local K^+ or Ca^{2+} concentrations at a distance x from the negatively charged membrane surface can be calculated by

$$K^+_{(x)} = K_b + \exp(-F\Psi_{(x)}/RT) \quad \text{---------- (3a)}$$
$$Ca^{2+}_{(x)} = Ca_b + \exp(-2F\Psi_{(x)}/RT) \quad \text{--------- (3b)}$$

where K_b and Ca_b are the bulk aqueous concentration of respective ions. F, R and T are Faraday constant, gas constant and absolute temperature, respectively. $\Psi_{(x)}$ is electrostatic potentials at distance x from the plane of charged bilayer and can be calculated by

$$\Psi_{(x)} = (2RT/F)\ln[(1+a\cdot\exp(-kx))/(1-a\cdot\exp(-kx))] \quad \text{---------- (4a)}$$

where k, the reciprocal of debye length (in Å) is

$$k = 1/(10(((\varepsilon\varepsilon_o RT)/(2F^2))/I)^{0.5}) \quad \text{---------- (4b),}$$

and

$$a = (\exp(F\Psi_{(0)}/2RT)-1)/(\exp(F\Psi_{(0)}/2RT)+1) \text{--(4c).}$$

Here, I is ionic strength and the surface potential, $\Psi_{(0)}$ in the presence of K^+ and Ca^{2+} was obtained by solving following Grahame equation (1947) of the surface charge theory of Gouy-Chapman-Stern,

$$\sigma = [\varepsilon\varepsilon_o RT\Sigma C_i[\exp(-z_i F\Psi_{(0)}/RT)-1]]^{0.5} \quad \text{----- (5)}$$

Where surface charge density (σ, charge/Å^2) was taken from Table 1. Equation (4a) strictly applies to the case where the solution contains only monovalent ions. But it could provide a valid estimate of the decay of potentials with distance where bulk Ca^{2+} concentrations are less than 0.1 mM such as the data shown in Figs 2 and 3 (Moczydlowski et al, 1985).

Estimation of surface charge effects by a rate theory model

Current-voltage data obtained at various K^+ concentrations were fit to double occupancy model of ion permeation by using the AJUSTE program (Alvarez et al, 1992) which also includes a simple Gouy-Chapman model of surface charge on the channel edges to simulate the effect of electrostatic potentials arising from membrane or protein ionizable groups. We used corrected current-voltage data based on the measured slope conductances for the calculation of energy profiles to minimize possible experimental errors caused by junction potentials and errors in adjusting ion concentration. The corrected data were obtained by the relation, I = g*V (Ohm's law) since the current-voltage relations of maxi K channel in this work were linear in the range of −50 − +50 mV (Fig. 2; Moczydlowski et al, 1985).

A 3-barrier-2-site (3B2S) model for the open state we used here includes six adjustable energy parameters expressed in RT units relative to a reference state of 55.5M: three peak energies (G1, G2, G3), two well site energies (U1, U2) and one ion-ion repulsion parameter A. The subscripts of parameters refer to positions with respect to the inside solution as shown in Fig. 6. The locations of peaks and wells are specified by six distance parameters, D1 through D6 as in Fig. 6, which have dimensionless units of fractional electrical distance and sum to 1.0. Six D parameters were fixed according Moss et al (1996) allowing central barrier to be located at an electrical distance of 0.5: D1=0.00, D2=0.35, D3=0.15, D4=0.15, D5=0.35 and D6=0.00. Two adjustable surface charge parameters of *cis* and *trans* sides, S_{cis} and S_{trans} are defined as the radius of a circle containing one electron charge (Alvarez et al, 1992). The quality of fit was evaluated visually, and also by the sum

square, which was defined as a weighted sum of squared differences between experimental and theoretical data minimized by the fitting routine (Alvarez et al, 1992). Ion activities of K^+ rather than molar concentration were used in all the computations for the energy profiles.

RESULTS

Single channel currents of a large conductance Ca^{2+}-activated K^+ (maxi K) channels from rat skeletal muscle were recorded at various holding voltages in a neutral and two negatively-charged bilayers. Neutral membranes were formed with phsphatidylethanolamine (PE) whereas negatively charged membranes were formed with either phosphatidylserine (PS) or phosphatidylinositol (PI). Fig. 1 illustrates the current fluctuations of single maxi K channels recorded at +20 or −20 mV. The open-state probability of maxi K channels recorded in the membranes formed with PS (80%) or PI (80%) were higher than those of the channels recorded in membranes formed with PE (100%). The single channel conductances were also larger in PS or PI membranes than in PE membranes. For example, the average slope conductances obtained from 13~23 membranes were 251±9.9, 360±8.7 and 356±12.4 (mean±SEM) pS at 100 mM KCl in PE, PS and PI membranes, respectively. However, there was little difference in the open-state probabilities and the conductances of the channel recorded in PS and PI membranes. Fig. 2 shows the current-voltage (I-V) relations at various symmetrical KCl concentrations (3~1,000 mM) in the various range of voltages. All I-V relations were linear and the slope conductances were larger in PS and PI membranes than in PE membranes. In addition, the conductance difference between neutral and negatively-charged bilayers was more notable at lower KCl concentrations.

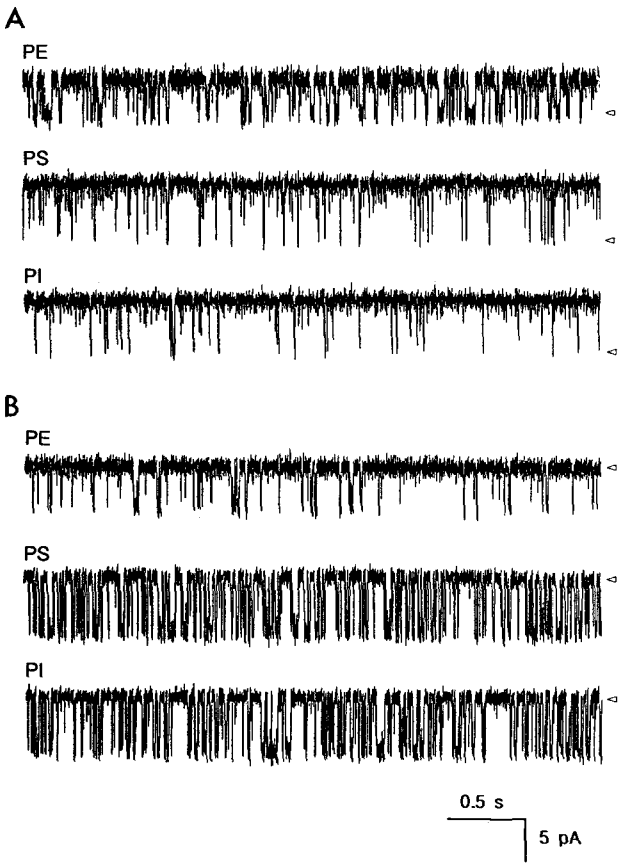

Fig. 1. Current records of a single maxi K channel from rat skeletal muscle in a neutral PE and negatively charged PS and PI bilayer at a holding voltage +20 mV (A) and −20 mV (B). Closed levels are indicated by open arrow heads. The buffer on both sides of membrane was 100 mM KCl, 10 mM HEPES-KOH, 0.5 mM EGTA, 0.6 mM $CaCl_2$, pH 7.4. Current records are filtered at 500 Hz and digitized at 2 kHz.

It is likely that the negative charges of phospholipid head groups attract cations, resulting in local accumulation of K^+ or Ca^{2+} ions near the membrane surface. If the binding sites for these ions on the channel protein are near enough to the lipid surface, apparent K^+ or Ca^{2+} concentration actually sensed by a channel protein will be greater than the ion concentration in the bulk solution. Thus, the gating equilibrium will be shifted toward open states because of higher local Ca^{2+} concentration. The K^+ conductances through the channel will be larger, if the pore is not saturated by K^+, because of higher local K^+ concentration. To estimate the possible effect of surface charge on the conductance and gating of maxi K channels, we analyzed the conductances as a function of K^+ concentration and the probability of opening as a function of Ca^{2+} concentration and membrane voltage in PE, PS and PI membranes (Moczydlowski et al, 1985).

Effect of negative surface charge on channel conductances

Fig. 3 shows the average slope conductances, obtained from individual I-V relations as shown in Fig. 2, as a function of K^+ concentrations in the

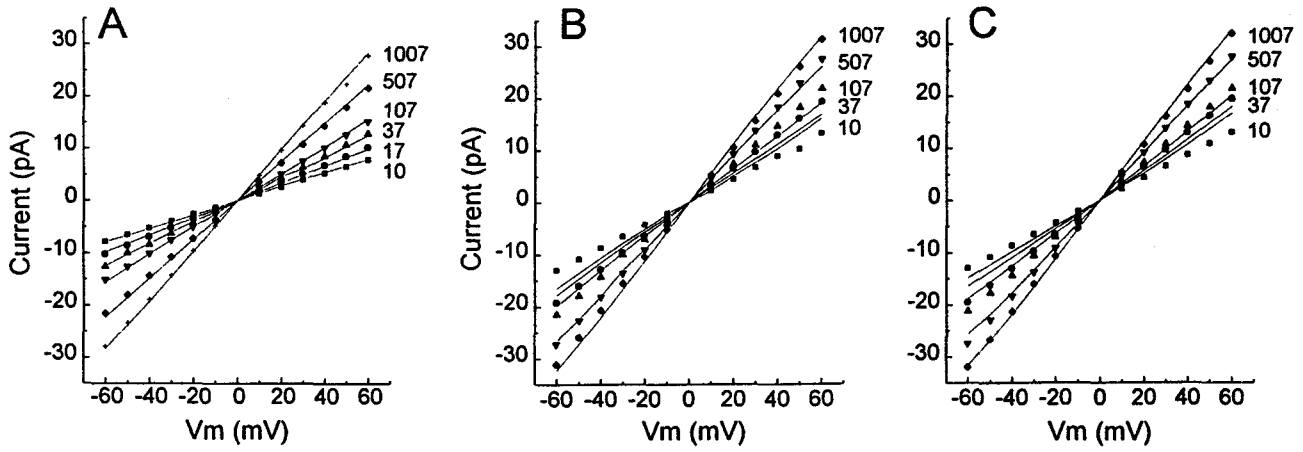

Fig. 2. Current-voltage relations for single maxi K channels recorded in phosphatidylethanolamine (PE, A) and negatively charged phosphatidylserine (PS, B) and phosphatidylinositol (PI, C) at various symmetrical KCl. Symbols represent the corrected conductance values based on the measured mean slope conductances as described in the materials and methods. Continuous lines are drawn by the best-fit parameters of the 3 barrier-2-site model for PE, PS and PI membranes as shown in the 2nd, 3rd and 4th columns of Table 2 respectively, assuming that the membrane surface charges do not change the energy profiles. Numbers indicate respective bulk KCl concentrations in mM.

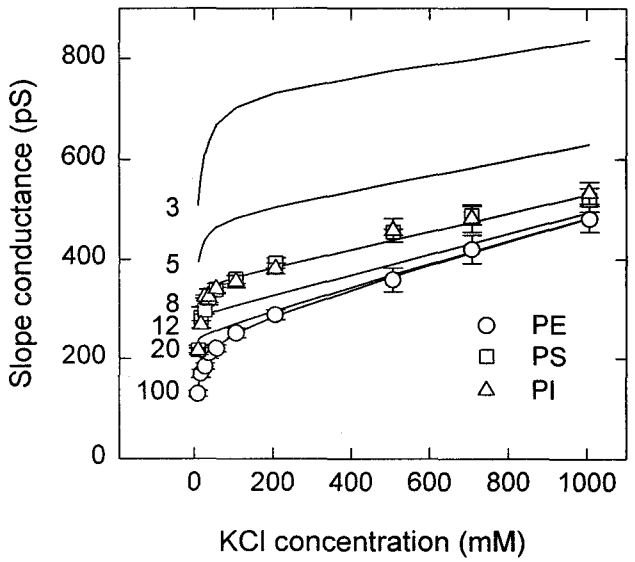

Fig. 3. Single channel conductances as a function of KCl concentration. Each symbol represents the mean slope conductances measured in PE, PS and PI at given KCl concentration (n = 8~10). The relations of conductance and K concentration can be well simulated by the following equation; conductance (g) = $\{G_1/(1+K_1[K^+])\} + \{G_2/(1+K_2/[K^+])\}$ as described in the materials and methods. Best-fit parameters were G_1 = 242 pS, K_1 = 8.7 mM, G_2 = 2,806 pS, K_2 = 10.6 M. The solid lines labeled 3, 5, 8, 12, 20 and 100 Å are computer fits expressing the local K^+ concentration at various distances from the surface of PS and PI bilayers, according to Gouy-Chapman-Stern theory in different K^+ concentrations.

membranes composed of 3 different phospholipids. In general, the conductances of maxi K^+ channels at low K^+ concentrations increased rapidly with the increase of K^+ concentration, but at higher K^+ concentrations it seemed to be saturated. First, we tried to relate the conductances of maxi K channels in neutral membrane to the bulk K^+ concentrations. We were not able to fit the relation of conductance-K^+ concentration of maxi K channels by a simple Michaelis-Menten equation as suggested by Lattore and Miller (1983). Instead, we could describe the conductance-K^+ relation by the following equation with a sum of two Langmuir isotherms as used by Moczydlowski et al (1985):

$$g = \{G_1/(1+K_1/[K^+])\} + \{G_2/1(1+K_2/[K^+])\} \text{ ----- (6)}$$

In this empirical equation, the variation of channel conductances, g is expressed as a function of K concentration where G_1 and G_2 are the maximum conductances and K_1 and K_2 are concentrations at half-saturation of the slope conductances for the high- and low-affinity terms. Best-fit parameters obtained by a non-linear curve fitting of the data obtained in neutral membranes to Equation (6) are G_1 = 242 pS, K_1 = 8.7 mM, G_2 = 2,806 pS and K_2 = 10.6 M. The solid line through the PE data in Fig. 3 was drawn by using these parameters, indicating that the empirical relation can closely simulate the experi-

mental data. For the conductance-K^+ relation in the negatively-charged membrane, one can simply assume that the negative charges on the surface of membranes attracted K^+ ions nearby ion binding site of maxi K channels and the local accumulation of K^+ ions caused to increase the current through the channel. Since the local K^+ concentration at a given distance from the membrane surface can be calculated by the surface charge theory, as described in Materials and Methods (Equations 3~5), it could be possible to simulate the conductances of maxi K channels in charged bilayers by incorporating the predicted local K^+ concentrations at the channel mouth to the relations of conductances vs K^+ as described in the above empirical equation. The solid lines in Fig. 3 represent the relations for the predicted channel conductances vs the local K^+ concentrations in PS or PI membranes, assuming the distance between the channel mouth and the membrane surface to be 3, 5, 8, 12, 20 and 100 Å. The conductance-K^+ relations obtained in PS and PI membranes were almost identical at all bulk K^+ concentrations tested and reasonably well corresponded to the predicted conductance values at a distance of 8 Å from the membrane surface. The results suggest that the channel sensed less than the full surface potential expected at the nearest distance from the membrane (d = 0 Å).

Effect of negative surface charge on channel gating

We observed that the open state probability (P_o) of maxi K channels at a fixed Ca concentrations varied widely as indicated by Moczydlowski et al (1985). Such heterogeneity in the P_o of maxi K channel was seen regardless of bilayer compositions. However, the voltage dependence of the probability of channel opening was similar in three types of membranes containing PE, PS or PI. The voltage corresponding to e-fold change in open-state probability was 12.8± 0.47 mV (mean±sem, n=11) in PE membranes.

Despite the heterogeneity of Ca^{2+} sensitivity in individual channels, the comparison of the average data compiled from single channels recorded at 3~4 different Ca^{2+} concentrations revealed a significantly higher Ca^{2+} sensitivity in PS and PI membranes than in PE membranes as shown in Fig. 4. At all Ca^{2+} concentrations tested, the P_o-V curves in PS and PI membranes shifted left along the voltage axis by 23 ~52 mV (40±4.7 mV, n=6). To analyze such data,

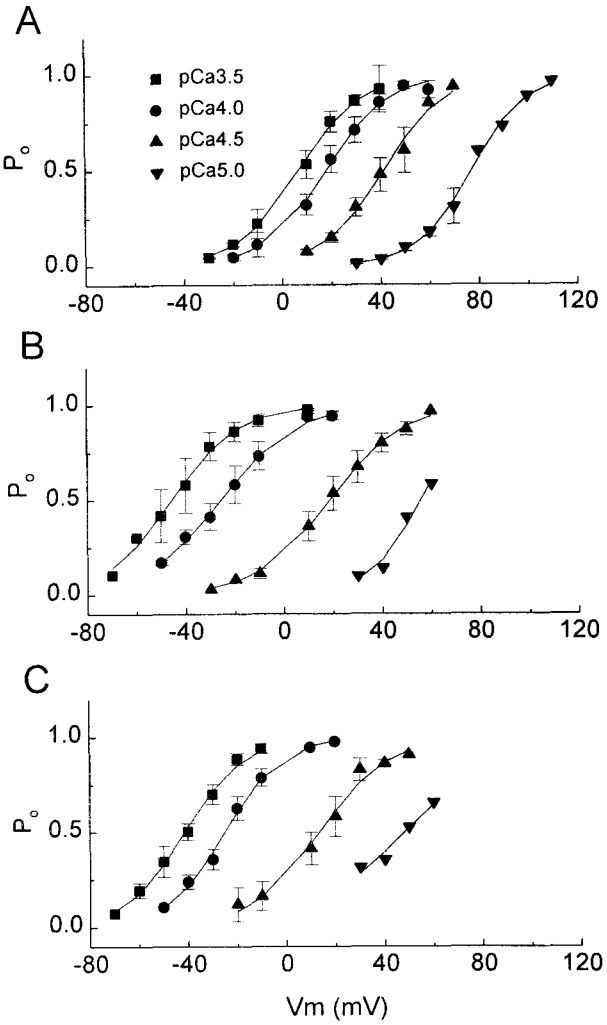

Fig. 4. Open state probability of maxi K channel in different Ca^{2+} concentrations in PE (A), PS (B) and PI (C). Symbols are means of 7 to 10 experiments and bars are standard errors. Solid lines are computer fits to equation, $P_o = 1/(1+\exp(-K(V-Vo)))$, where K is the slope factor and Vo, membrane potential at P_o=1/2 as described in the materials and methods.

we first plotted the voltages at half-saturation (V_o) where P_o is 0.5, at the Ca^{2+} concentrations of 30, 100 and 300 μM in PE, PS and PI bilayers (Fig. 5). The expected local Ca^{2+} concentrations of PS and PI membranes at V_0 = 0 mV were to be 5.9 and 6.4 fold higher than that of PE membranes. The calculations based on the surface charge theory described in Materials and Methods indicate that such accumulation of local Ca^{2+} concentrations can be caused by the surface potentials sensed at a distance of 8.97 and 8.73 Å from the surface of PS and PI membranes, respectively. These results suggest that

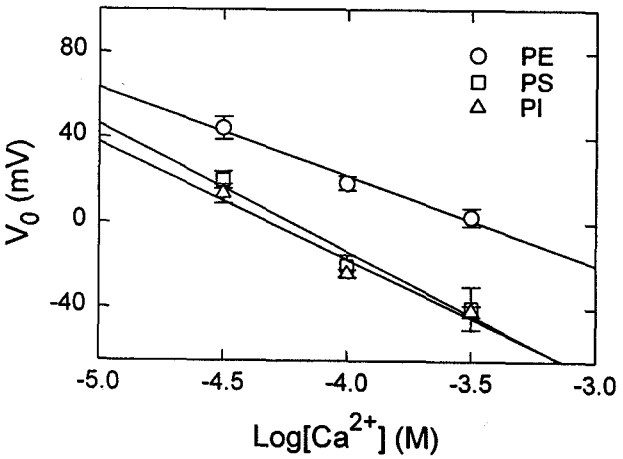

Fig. 5. Ca^{2+} dependence of Vo, the voltage at which channels are open half of the time in PE, PS and PI bilayers. Symbols are means of 7 to 10 experiments and bars are standard errors. Lines were drawn by linear regression and the slopes are −41.4, −59.7 and −54.9 mV/log[Ca^{2+}](M) for PE, PS and PI membranes, respectively. Three slopes were not statistically different ($0.05<p<0.2$). The respective log[Ca^{2+}] values at V_0 = 0 mV are −3.45, −4.22 and −4.26 mV for PE, PS and PI membranes.

the Ca^{2+} activation of maxi K channels was in progress at a lower Ca^{2+} concentration than expected by the surface potentials. The surface potentials was about −160 mV at 10 mM KCl and 0.1 mM $CaCl_2$ in both PS and PI membranes.

Energy profiles and surface charge

Ion permeation through a channel can be described by applying Eyring rate theory to a linear chain of ion binding sites as a discrete kinetic model for ion transport (Hille, 1992; for review see Eisenman & Horn, 1985). We used a similar method described by Alvarez et al (1992) to obtain the best-fit energy profile for I-V data of single maxi K channels. For the I-V data obtained in PE membranes (Fig. 2A), the solid lines were drawn according to the best-fit parameters and are closely predicting the experimental data points. The energy profiles for K^+ permeation through maxi k channels in PE membranes were illustrated in Fig. 6. In this calculation, the surface charge values in *cis* and *trans* side of the membranes were fixed to one charge per circle with radius of 90 Å. Such charge density, obtained by the fitting routine, is reflecting a negligible amount of surface charge in PE membrane as expected. The

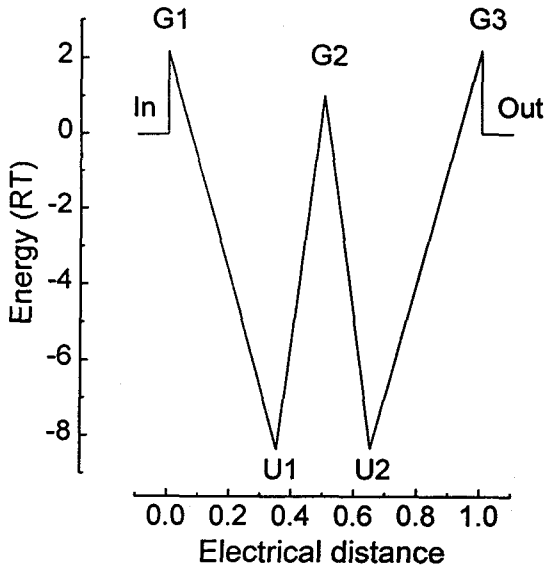

Fig. 6. Apparent energy barrier profiles for K^+ permeation through maxi K channels from rat muscle in PS membranes with parameters shown in Table 2 (the 2nd column). Best-fit parameters were obtained by fitting the current-voltage data obtained at various K^+ concentrations with a 3-barrier-2 site double occupancy model of ion permeation as described in the materials and methods.

Table 2. Best fit parameters of the 3B2S double occupancy model for K^+ in 3 phospholipid bilayers

Parameters	PE	PS*	PI*
G1	2.19±0.063	2.19	2.19
G2	1.00±(fixed)	1.00	1.00
G3	2.20±0.053	2.20	2.20
U1	−8.35±0.071	−8.35	−8.35
U2	−8.33±0.070	−8.33	−8.33
A1	2.20±0.017	2.20	2.20
S_{cis}	90	18.55±0.56	15.48±0.79
S_{trans}	90	16.01±0.86	18.84±0.47
Sum square	0.58	8.82	8.13

*All the parameters except for surface charges were fixed to those in PE membranes. G1-G3, energy levels in RT units of three peaks from internal side; U1 and U2, two well energy levels; A1, energy for ion-ion interaction; S_{cis} and S_{trans}, surface charge density of internal and external side of bilayer as the radius of circle containing one electron charge in Å.

small sum square (0.58) and excellent simulation of experimental data (solid line in Fig. 2A) indicate a good quality of fit. Assuming the energy profiles for ion permeation in charged membranes were not changed (Bell & Miller, 1984), we attempted to calculate the surface charge density that could increase the conductances of maxi K channels as much as shown in I-V data obtained in PS or PI membranes. The resulting best-fit parameters are listed in the 3 rd and 4 th columns in Table 2. The predicted charge densities were about one tenth of the measured surface charge density shown in Table 1 in both PS and PI membranes (0.11 charge/nm^2). Although the decay of electrostatic potentials varies with ionic strength, the preferred distance from the surface that can result in such a reduction in effective charge density (or surface potential) was calculated to be 7 and 9 Å at the K^+ concentration ranges of 200 to 10 mM. Such insulation distances correspond to the effective surface potentials of -31 and -86 mV respectively. With the resulting parameters (3 rd and 4 th columns in Table 2), the experimental data could be simulated though the result was not as good as those for the data in PE membranes (Fig. 2, PS and PI).

DISCUSSION

Surface charge and maxi K channel behavior

Estimation of the effect of surface charge from the conductance and gating of maxi K channels by calculations based on the surface charge theory (Bell & Miller, 1984; Moczydlowski et al, 1985; Lattore et al, 1992) indicates that maxi K channels sense the surface potentials at a distance of 8~9 Å from the membrane surface (Figs. 3 and 5). In addition, the surface charge density predicted by a 3-barrier-2-site model of energy profile for the permeation of K ions suggested that channel mouth is located or insulated by 7~9 Å from the fixed charge of the bilayers formed with PS or PI (Fig. 2 and Table 2). Although our calculations were not based on the detailed structural information of the channel in the lipid bilayer, the inferred distances estimated from the conductance, gating and energy profiles of maxi K channels are consistent.

The activity of a membrane protein can be dependent on the property of membrane phospholipid (Carruthers & Melchior, 1986; Green & Andersen, 1991). Phosphatidylserine (PS) has been widely used as standard phospholipid in forming planar lipid bilayers to record single channel currents (Miller, 1986). For phosphatidylinositol (PI), to the best of our knowledge, the present study is the first to incorporate any ion channel into bilayers formed with PI. In addition to serving as the source of intracellular second messengers such as inositol trisphosphate (Berridge, 1984), PI can exert electrostatic effect on the charged particles around because it has net one negative charge like PS. Under our experimental conditions, PI bilayers were larger in size and their 'thinning' was faster than PS bilayers, indicating higher fluidity of PI membrane. In addition to the differences in the head group structure and fluidity, PI and PS membranes may impart other properties because of the subtle variation. PS used was a synthetic lipid of high purity with defined acyl chain length (16-0, 18-1). PI used was extracted from bovine liver, and its acyl chain length may not have been homogeneous, and therefore different from that of PS. It was also known that binding of Ca^{2+} to PI was different from that to PS (Hayashi et al, 1984). Despite such differences in their structure and fluidity, the observed behaviors of maxi K channels in two phospholipid bilayers were strikingly similar in all the figures shown in the results. These facts strongly suggest that the enhancement of the conductance and gating of maxi K channels in both cases are mostly due to the one negative charge in the head group. Another source of surface charge could be the maxi K channel protein itself (Mckinnon et al, 1987), but since other experimental conditions except the bilayer composition are identical, the effect of surface charge from the channel protein could be mostly nulled out as in the classical experiments with isolated tissues in pharmacology (Kenakin, 1989).

Origin of differential sensitivity to the membrane surface charge of K^+, Na^+ and Ca^{2+} channels

Since the informations on the structure of maxi K channels are not complete enough to understand the full geometries of ion channels in the lipid membrane, any physical interpretation of the insulation distance could be erroneous. Available data indicate that the inferred insulating distances in two types of K^+ channels are 8~9 Å (Bell Miller, 1984;

Moczydlowky et al, 1985; this work). However, those in Ca^{2+} and Na^{+} channels are 20 and >20 Å, respectively (Coronado & Affolter, 1986; Worley et al, 1992), suggesting negligible effects of membrane surface potential on the channels. It is not yet understood what makes such a difference in the sensitivity of ion channels to the membrane surface potentials. In case of acetylcholine receptors, the structure determined by X-ray crystallography demonstrated that acetylcholine receptor molecule (MW 290 kDa) extended its structure about 20 Å beyond the membrane into the cytoplasmic medium and 60 Å into the extracellular medium (Kisteler et al, 1982; Toyoshima & Unwinn, 1988). Therefore, it is possible that large membrane proteins such as Na^{+} (MW ~290 kDa) or Ca^{2+} channels (MW ~447 kDa, Hille, 1992) have an extramembrane extrusion longer than 20 Å and their activities are insensitive to membrane surface charge if the ion conducting pathway is located in it. Based on similar reasoning, Cornnado & Affoltor (1986) have suggested that t-tubule Ca^{2+} channels were probably large molecules. Similarly, we could interpret that SR-K and maxi K channels are more sensitive to the effect of membrane surface charges because of their molecular structure were much smaller than those of Na^{+} and Ca^{2+} channels. However, recent works revealed that maxi K channels are also large molecules and comparable to Na^{+} or Ca^{2+} channels. Each functional unit of a maxi K channel has tetrameric structure of 4 individual channel proteins of 1184 amino acids (Aktinson et al, 1994; Shen et al, 1994). In addition, it was also known that the purified maxi K channels from smooth muscle were composed of 62 kDa α subunit and a 31 kDa β subunit (Garcia-Calvor et al, 1994). These evidence clearly indicate the macromolecular nature of maxi K channels. Therefore, the higher sensitivity of maxi K channels to membrane surface potential is more likely due to its geometrical property in the lipid membrane. An important difference of K^{+} channels from Na^{+} or Ca^{2+} channels is that functional Na^{+} and Ca^{2+} channels are composed of single protein of multiple subunits but a functional unit of K^{+} channels including maxi K channel are composed of four small independent channel proteins (Hille, 1992). Therefore, it is possible that such assembly of maxi K channel can allow a closer access of membrane phospholipids to the channel mouth so that the surface potential can be better sensed by maxi K channel.

These results demonstrate that the conductances and open-state probability of maxi K channels are enhanced in the bilayers formed with phosphatidylserine and phosphatidylinositol. It is suggested that the electrostatic potential from negative surface charges of bilayer are mostly responsible for such behavior of maxi K channels in charged bilayers.

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