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Properties of Single K^+ Channels of Skeletal Muscle Incorporated into Planar Lipid Bilayer

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=ABSTRACT=

Single K^+ channels of skeletal muscle from the rat and frog were incorporated into planar lipid bilayers and their properties were studied. Fusion was induced by an osmotic gradient. Of the four types of K^+ channels recorded, the two most frequently observed were a voltage and Ca^{2+}-activated K^+ channel and a K^+ channel with a prominent conductance substate. The first K^+ channel was identified as the large Ca^{2+}-activated K^+ (BK) channel because the open-state probability was increased with depolarization (e-fold change per 10.6±3.5 mV, n=8) and internal Ca^{2+} (half-activation at 16.7±3.8 mV, n=8, pCa 4) and its conductance was large (247±4.9 pS, n=24 in 0.1 M KCl). Lifetime distributions of open- and closed-states could be fitted with single exponentials of several milliseconds. The mean open- and closed-lifetimes were linearly dependent on the intracellular $[Ca^{2+}]$ and $1/[Ca^{2+}]$, respectively.

The second K^+ channel showed a conductance substate at 30~60% of the open state. Its current-voltage relation was linear in the range of −80 ~ +80 mV. The slope conductance of the substate and open-state were 40 and 144 pS in 0.2 M KCl, respectively. The channel was highly selective for K^+ over Cl^-. The open-state probability was weakly voltage-dependent (e-fold change per 35 mV). The lifetime distributions of open- and closed-states were fitted with two exponentials and the major gating occurred slowly at several hundred milliseconds. Based on the above results, we think the second type of K^+ channel is the sarcoplasmic reticulum K^+ (SRK) channel. In addition, both types of channel were also incorporated into the lipids extracted from the skeletal muscle. The channel properties recorded in the bilayers formed from synthetic and extracted lipids were qualitatively similar.

Our data indicate that BK and SRK channels are rich in the skeletal muscle and their properties and regulation could be effectively studied in planar lipid bilayer.

Key Words: Skeletal muscle, Large Ca^{2+}-activated K^+ channel, Sarcoplasmic reticulum K^+ channel, Planar lipid bilayer

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INTRODUCTION

K^+ channels play important roles in controlling electrical excitability in physiological and pathological states of skeletal muscle. Multiple types of skeletal muscle K channels have been characterized: 1) inward rectifier K^+ channels that activate with hyperpolarization and inactivate with depolarization (Rowe et al, 1990), 2) delayed rectifier K^+ channels that activate with depolarization and contribute to membrane repolarization during the action potential (Standen et al, 1985), 3) large conductance Ca^{2+}- activated K^+ channels (BK channels) which are highly conductive (200~400 pS) and sensitive to internal Ca^{2+} and voltage (Barrett et al, 1982; Moczydlowski & Latorre, 1983), 4) ATP-sensitive K^+ channels that are inhibited by internal ATP (Spruce et al, 1985), and 5) sarcoplasmic reticulum K^+ channels which show a typical subconducting state (Hill et al, 1990; Uehara et al, 1991) and are considered as the route of K^+ influx during Ca^{2+}-release from sarcoplasmic reticulum (Caille et al, 1985; Abramcheck & Best, 1989).

The expression of the type and density of K^+ channels are different depending on the developmental or pathological state. For instance, small conductance Ca^{2+}-activated K^+ channels, normally not expressed in adult skeletal muscle, are expressed after denervation in non-innervated myocytes in culture and in vivo during the embryonic period (Schmidt-Amtoarchi et al, 1985; Escorbar et al, 1993). In muscular dystrophy, BK channels become less sensitive to Ca^{2+} which may explain the elevated resting Ca^{2+}-level in the patient (Rowe et al, 1990). Such changes in the properties and regulatory pathways of the ion channels have been effectively studied from the analysis of single ion channels or of collective whole cell current recorded by using the patch clamp technique (Hamill et al, 1981) and the planar lipid bilayer method (Miller, 1986).

In the present work, we characterized the K^+ channels of skeletal muscle by using the bilayer method which allowed us to access the ion channels present in sarcolemma as well as sarcoplasmic reticulum with relative ease. Two most frequently recorded channels were the large conductance Ca^{2+}-activated K^+ and sarcoplasmic reticulum K^+ channels. In addition, we incorporated such channels in bilayers formed from membrane lipids extracted from the skeletal muscle. The preliminary results of this work have been communicated previously (Ryu & Park, 1992).

MATERIALS AND METHODS

Microsomal preparation

Native membrane vesicles from whole muscles of hind limbs of the rat (Sprague-Dawley, 200~300 g) or bullfrog (200~300 g) were prepared according to procedures modified from Guo et al (1987). The dissected tissue was minced in 0.3 M sucrose buffer (10 mM HEPES, 0.2 mM EDTA, 3 mM NaN_3, pH 7.4 adjusted with 2 N NaOH) at 0~4℃. The muscle was divided into batches of 100 g and minced into small pieces (< 0.5 × 0.5 × 0.5 cm) with scissors. The minced tissue was combined with 400 ml of 0.3 M sucrose buffer containing 0.3 mM phenylmethylsulfonylfluoride (PMSF) and homogenized for 30 seconds at high speed in a blender. Such homogenization was repeated three times at 30-second intervals. The homogenate was centrifuged for 30 minutes at 2,500 xg, the supernatant was filtered through four folds of gauze, and solid KCl was added to a final concentration of 0.6 M. The suspension was then stirred for 30 minutes at 0~4℃ and centrifuged at 100,000 xg for 40 minutes at 2℃. The pellets

were resuspended using a glass/glass homogenizer in 0.3 M sucrose buffer in a final volume of 50～100 ml. This suspension was centrifuged at 10,000 xg for 30 minutes and the supernatant was overlaid on a discontinuous sucrose gradient consisting of 20, 30 and 40% (w/w) sucrose buffer and centrifuged overnight at 85,000 xg. Microsomes banding in the 20/30% and 30/40% sucrose interface were collected, diluted 2～3 times with distilled water and pelleted at 100,000 xg for one hour. The pellets were resuspended at 0.5～10 mg of protein/ml in 10% sucrose, 10 mM HEPES, 0.2 mM EDTA, pH 7.4 (adjusted with 2 N NaOH) and stored in small aliquots at −80℃.

Extraction of membrane lipid

Membrane lipids were extracted from whole muscles of the rat hind limb (200～300 g) by the method adapted from Wood et al (1989). The dissected tissue (100 g) was minced, homogenized in 600 ml of 0.3 M sucrose, 10 mM HEPES, pH 7.2 (adjusted with 2 N KOH) and centrifuged at 700 xg for 30 minutes. The supernatant was centrifuged at 14,000 xg for 40 minutes and the pellets discarded. The supernatant was further centrifuged at 42,000 xg for one hour. The resulting pellets were vigorously shaken in 4 volumes of chloroform- methanol 2:1 (v/v) and centrifuged at 750 xg for 10 minutes. The lower organic phase was removed and placed in a separate tube. The upper layer was extracted again with 2 volumes of acidified chloroform-methanol (4:1, v/v) containing 26 mM HCl and centrifuged. The latter acidic organic layer was neutralized with NH_4OH and combined with the neutral organic extract. The combined extract was partially evaporated under a stream of N_2. The concentration of phospholipid was dertermined by measuring total inorganic phosphate (Ames, 1966)

Planar bilayers and channel incorporation

Planar bilayers were formed from a solution of palmitoyloleoylphosphatidyl-ethanolamine (POPE), POPE/palmitoyl-oleoyl-phosphatidylcholine(POPC) (70/30%), or extracted phospholipids in decane (25 mg/ml) by a painting method (Moczydlowski & Latorre, 1983). Synthetic lipids were purchased from Avanti Polar Lipids (Birminghamn, AL, USA). Bilayer was formed according to the following procedure. A drop (1～2 μl) of bilayer forming solution was applied to the edges of the aperture (20 μm) in a polystyrene cup and allowed to air dry (conditioning). Then, both sides of the aperture were filled with buffer solution (10 mM HEPES, pH 7.2, adjusted with 2 N NMDG, N-methyl D-glucamine). Bilayer forming solution was applied over the aperture using a glass rod fashioned from a fire-polished capillary pipette. The formation and size of the bilayer was estimated by monitoring the rectangular capacitative current of the bilayer induced by triangular voltage pulses. After a stable membrane of 70～150 pF was formed, the ionic gradient was established by adding appropriate amount of 3 M KCl and the membrane preparations (1～5 μl) were added to *cis* side. The side of bilayer to which the membrane preparations were added is defined as *cis* (1.2 ml) and the other side of the bilayer as *trans* (0.6 ml). The channel incorporation was attempted in a 40/200 mM or in a 0/100 mM KCl gradient (*trans/cis*) at 0 mV under continuous stirring with a small magnetic bar (2～3 mm). The incorporation of channel proteins was observed as an abrupt appearance of single channel activity on the oscilloscope screen. For incorporation of Ca^{2+}-activated K^+ channels, 100 μM of free Ca^{2+} was added to the *cis* buffer solution.

Electrical measurement and data analysis

Currents were measured at constant voltage with

a bilayer amplifier (BC 525A, Warner Instr. Co., Hemden, CT, USA). The amplifier headstage was connected with two Ag/AgCl wire electrodes to two separate wells containing 0.5 M KCl. These wells were connected to the two bilayer chambers with small U-shaped salt agar bridges formed from capillary pipettes that were filled with 2% agar in 0.2 M KCl and 1mM EDTA. The electrode asymmetry and junction potentials were corrected at the beginning of each experiment in a symmetrical solution. Single channel currents at steady-state membrane potential were stored on VCR tape using a VR-10 data recorder (Instrutech, Elmont, NY, USA) for subsequent analysis and also recorded on paper by a two-channel pen recorder. The stored data was replayed through an 8-pole Bessel filter at 100~500 Hz (−3 db corner frequency) and digitized at 2~20 kHz on to a 486 PC for display and analysis using a TL-1 Labmaster (125 kHz) and pClamp programs (Axon Instrument, Foster City, CA, USA). Parameters of single channel activity such as amplitude, open-state probability, dwell-time distribution and time constants were obtained with the aid of the analysis software (pClamp, Ver 6.0).

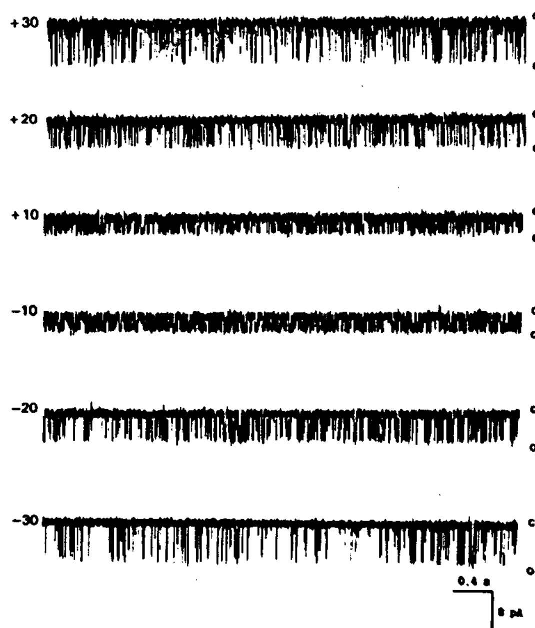

Fig. 1. Current records of a single Ca^{2+}-activated K^+ channel from rat skeletal muscle incorporated into a phosphatidylethanolamine bilayer. Membrane potentials applied across the bilayer are shown on the left and the current levels of open (o) and closed-states (c) are shown on the right. The solution on both sides of the bilayer contained 100 mM KCl, 10 mM HEPES-KOH (pH 7.2), 0.5 mM EGTA and 0.6 mM $CaCl_2$. Current records are filtered at 500 Hz (−3 db, corner frequency 8-pole Bessel filter) and digitized at 2 kHz.

RESULTS

Types of skeletal muscle K^+ channels observed in planar lipid bilayer

Four types of K^+ channels were recorded from the microsomes of skeletal muscles. Among these, large Ca^{2+}-activated and sarcoplasmic reticulum K^+ channels were readily incorporated into the phospholipid bilayer and characterized in detail. Although both channels were observed from the microsomal fractions of either 20/30 or 30/40% sucrose interfaces, large Ca^{2+}-activated K^+ channels were more frequently obtained from the vesicles of 20/30% sucrose interface but sarcoplasmic reticulum K^+ channels were from those of 30/40% sucrose interface. The other two types of K^+ channels observed were ATP-sensitive K^+ channels (Ryu, 1992) and 40 pS K^+ channels with flickering activity and little sensitivity to voltage. The incorporation of the latter K^+ channel was not frequent enough to allow detailed characterization.

Large Ca^{2+}-activated K^+ channels

Large Ca^{2+}-activated K^+ channels were identified by their large conductance and sensitivity to voltage and internal Ca^{2+}. Fig. 1 shows the current of a large Ca^{2+}-activated K^+ (BK) channel recorded in

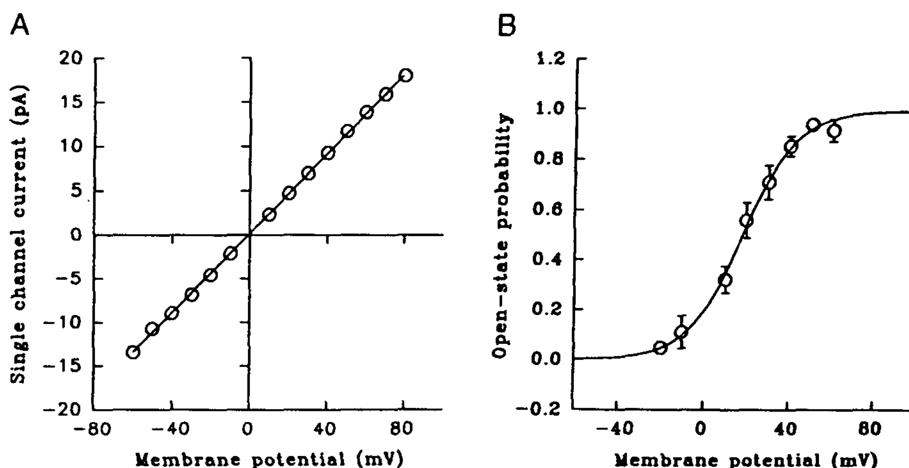

Fig. 2. Voltage-dependence of the conductance and activity of a Ca^{2+}-activated K^+ channel of the rat skeletal muscle. A, current voltage relations of a Ca^{2+}-activated K^+channel. The slope conductance around 0 mV is 230 pS. B, voltage dependence of open-state probability of Ca^{2+}-activated K^+ channels of rat skeletal muscle. Symbols and bars are means and standard errors from experiments. Solid lines are drawn using best-fit parameters of the Boltzman Equation, $P_0 = 1/(1+exp(-k(V-V_{1/2})))$. The voltage at half activation (V_0) is 18 mV and slope factor (k) is 0.0789.

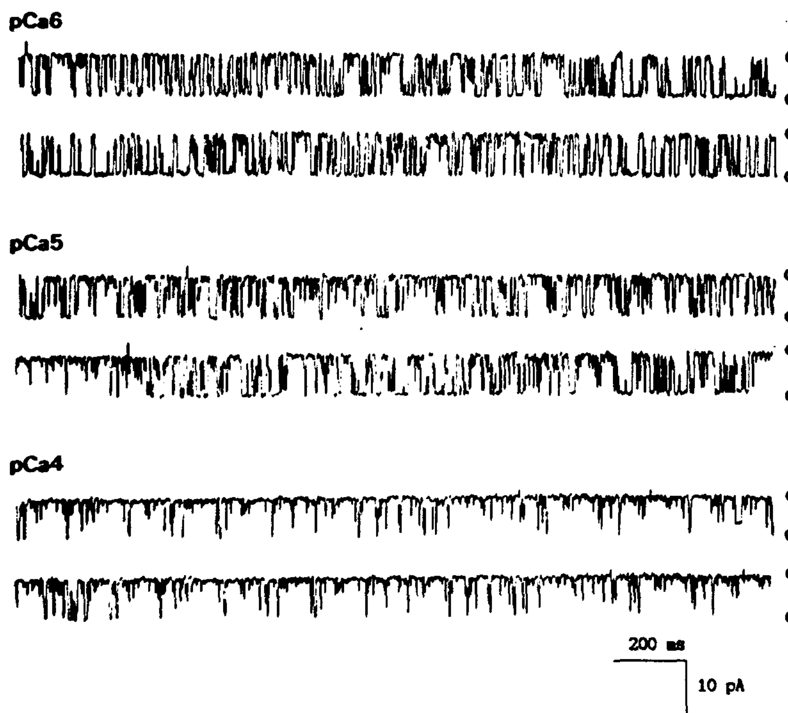

Fig. 3. Current records showing a BK channel's activity at different Ca^{2+} concentrations. The channel activity was recorded at 0 mV in the presence of 150 mM external NaCl and 150 mM internal KCl. Free Ca^{2+} concentration was adjusted by adding $CaCl_2$ or EGTA according to Fabiato (1988).

a solution containing symmetrical 100 mM KCl and 100 μM free Ca^{2+}. The open-state probability increased with depolarization and decreased with hyperpolarization. The orientation of the incorporated BK channels were determined by their voltage- and Ca^{2+}-sensitivities. The channels were consistently incorporated with internal side toward *cis* chamber, indicating that the vesicles had inside-out configuration. The current-voltage relation was linear in the range of $-80 \sim +80$ mV (Fig. 2A). The slope conductances ranged from 197 to 398 pS at symmetric 100 mM KCl. Average conductance of BK type channels was 247 ± 4.9 pS (mean ± s.e.m., n=24). Among these, the most frequently observed were the channels with about 210 pS (n=8). Fig. 2 shows that open-state probability of BK channels increased with membrane depolarization ($n=4 \sim 9$). The half-activation voltage was 16.7 ± 3.8 mV (n=8) at internal 100 μM free Ca^{2+} in cytoplasmic side. The potential needed for e-fold increase in open-state probability was 10.6 ± 3.5 mV (n=8) which is similar to those of other BK channels (Reinhart et al, 1989; Toro et al, 1990). BK channels are well known to be activated by cytoplasmic Ca^{2+} (Barrett et al, 1982; Moczydlowski and Latorre, 1983). Fig. 3 shows the BK channel activity at 0 mV at three different Ca^{2+} concentrations. With increasing Ca^{2+} concentration, the channel spent more time at open-state. The average open-state probability of two BK channels 0.59, 0.70 and 0.81 at pCa 6, 5 and 4, respectively. The estimated Hill coefficient was 0.24. Dwell-time distributions of open- and closed-states were compiled from 1-minute segments of single channel data which was filtered at 500 Hz (-3 db, 8-pole Bessel filter) and digitized at 20 kHz. At all Ca^{2+} levels, histograms of open-state dwell-times were well fitted with single exponentials. The histograms of closed-state dwell-times could be fitted one or two exponentials, but fits with double exponentials were not always

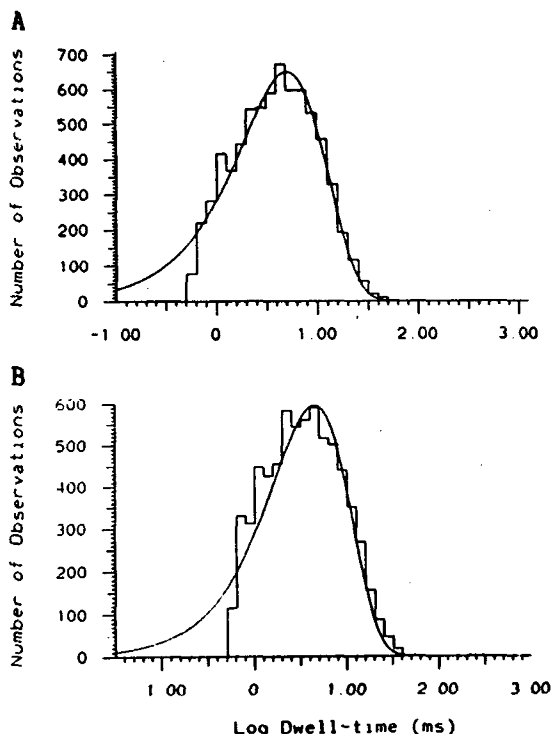

Fig. 4. Frequency density histograms of open- and closed-state dwell times. The channel activity was recorded at 0 mV, pCa 4 and 150 mM external NaCl/150 mM internal KCl. Dwell-time populations of open and closed events were compiled and analyzed after filtering at 500 Hz (−3 db) and sampling at 20 kHz. The ordinate represents the number of events per bin and a log-binned abscissa is shown. Both open- (A) and closed-state (B) dwell-time histograms were fitted with single exponentials and solid lines are single exponential fits with time constants of 5.3 ms (A, 7,069 events) and 4.7 ms (B, 6,765 events).

better than those with single exponentials (Fig. 4). The open-state lifetimes measured by the histograms shown in Fig. 4 are apparent lifetimes overestimated by the limitation of detecting very short closing or blocking events. When both open- and closed- dwell-times are fitted with single exponentials, the measured apparent open-state lifetimes can be corrected by the following cor-

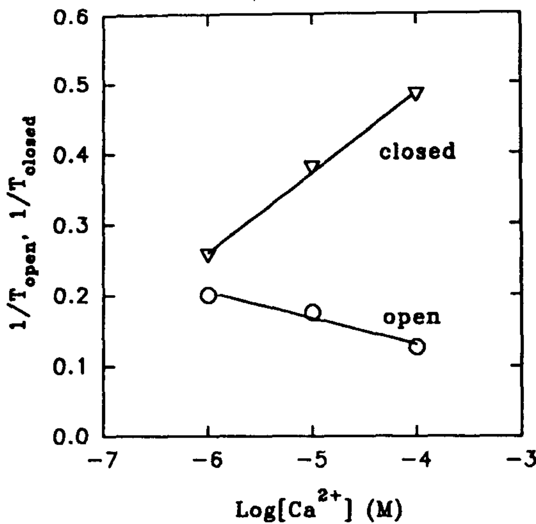

Fig. 5. Mean open- and closed-times as a function of internal Ca^{2+} concentration. The mean open- and closed-times were obtained by fitting the respective dwell time histogram as described in Fig 4. The slopes for open and closed times are 0.113 and −0.0385, respectively.

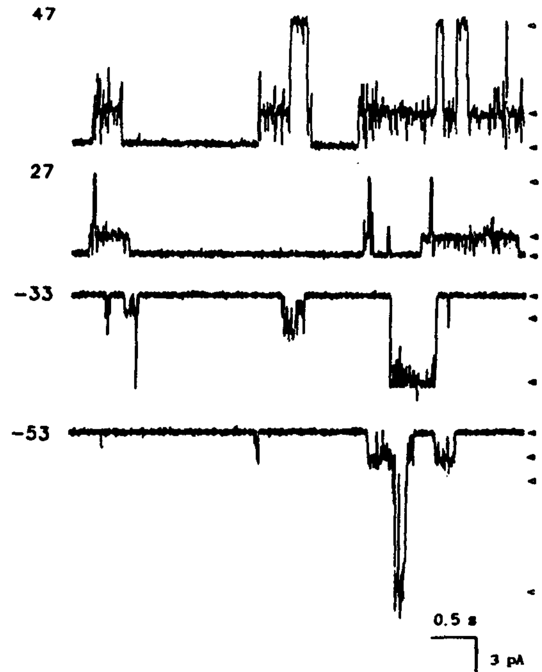

Fig. 6. Current records of a sarcoplasmic reticulum K^+ channel from frog skeletal muscle. Unitary currents were recorded in a solution containing 200 mM KCl, 1 mM EGTA, 10 mM HEPES-NMDG (pH 7.2). Membrane potentials are marked on the left and the zero-current level is marked with filled arrow head on the right. Note the substate of the channels.

rection,

$$\tau_o = \tau_{o,obs} * \exp(-\tau_d/\tau_c)$$

where τ_o is the corrected open-state lifetime, $\tau_{o,obs}$ is the open-state lifetime obtained by histogram fitting, τ_d is a limit of time resolution for a system which is estimated from $0.179/f_c$, where f_c is the corner frequency (Colquhoun and Sigworth, 1983), and τ_c is the fitted lifetime of the closed-state histogram. Fig. 5 illustrates that open-state dwell-times are linearly related to internal Ca^{2+} concentration while closed-state dwell-times are to $1/[Ca^{2+}]$. The slope for closed-state dwell times (0.113) was 3 times steeper than that for open-state dwell times (−0.037).

Sarcoplasmic reticulum K^+ channel

As reported in the previous works (Hill et al, 1990), sarcoplasmic reticulum K^+ (SRK) channels were identified by the presence of a substate of single channel current. We think the K^+ channel shown in Fig. 6 is the SRK channel because of its substate behavior and other unitary properties described below. The substate amplitude of this SRK channel from frog skeletal muscle was about one third of the fully open-state at 47 mV. Fig. 7 shows the current-voltage relations in 200 mM symmetric KCl and 40 mM external/200 mM internal KCl from 3∼4 experiments in each condition. The current-voltage curve was linear in the voltage range of −80 ∼ +80 mV and the slope conductances were 144 and 99 pS in 200 mM symmetric KCl and 40 mM external/200 mM

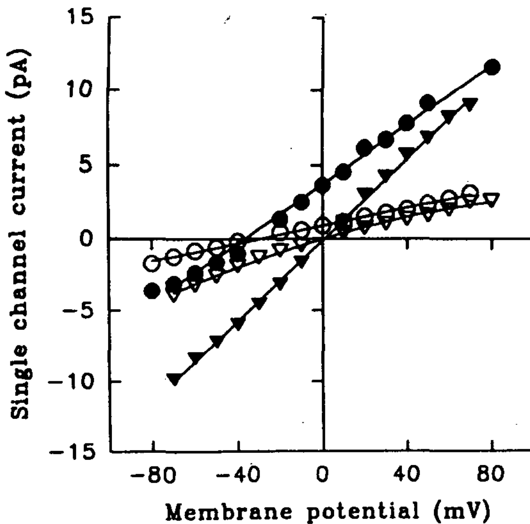

Fig. 7. Current-voltage relations of the sarcoplasmic reticulum K^+ channels from frog skeletal muscle. Current-voltage relations were obtained in 40 mM trans KCl and 200 mM cis KCl (circles) or symmetrical 200 mM KCl (triangles). Symbols are means from 3~4 experiments and error bars are not shown are when smaller than symbols. Open-states and substates are represented by filled and open symbols, respectively.

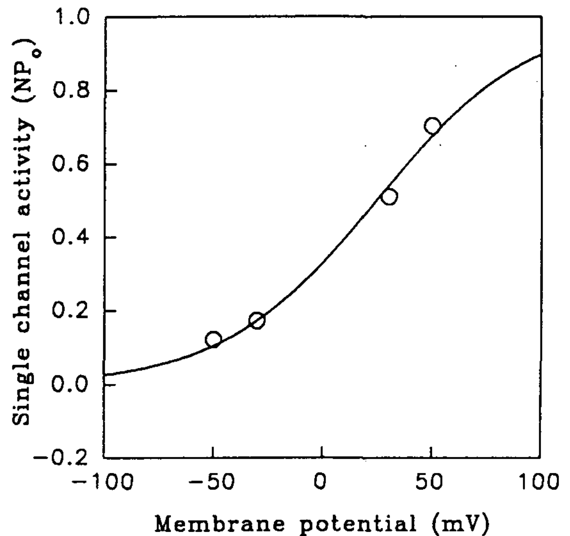

Fig. 8. Voltage dependence of sarcoplasmic K^+ channel from frog skeletal muscle. The activity of two SRK channels were measured from a 50~70 sec segment of records obtained in a phosphatidylethanolamine/phosphatidylcholine (7:3) bilayer and at symmetrical 200 mM KCl and 20 mV. The activity included both substate and open-state. Solid lines are drawn using best-fit parameters in the Boltzman Equation as described in Fig 2B. Slope factor (k) is 0.029.

internal KCl, respectively. The slope conductances of the respective substates were 40 and 27 pS which were at 28% of fully open-states. The reversal potentials at reduced external KCl (40 mM) shifted to -34 ± 3.9 mV (n=3) in the open-state and -30 ± 3.1 mV (n=4) in the substate, which are close to the calculated reversal potential of K^+-selective channel, -36.6 mV, indicating high selectivity for K^+ over Cl^-. Fig. 8 shows that the activity estimated from two functional SRK channels of frog skeletal muscle was weakly voltage-dependent. Most of the channel activity was contributed by the open-state and less than 5 % was by the substate in the voltage range -50 ~ $+50$ mV. The slope of the curve was much less steep than that of the BK channel. Fitting these data with the Boltzman Equation gave 35 mV for an e-fold change in channel activity. The activity of SRK channels was stable for up to 1 hour. Fig. 9 shows data from a 60-minute record of single SRK channels of the rat skeletal muscle that showed little change in activity during that period. The substate conductance of the rat SRK channel occured at about half that of the fully open-state, which was larger than that of the frog SRK channel (Fig. 6). Fig. 9B shows dwell-time histograms of the open-state, substate and closed state, which were fitted with two exponentials. The main and longer lifetimes of open- and closed-states were hundreds of milliseconds (about 500 ms), and minor and short lifetimes were about 6 ms. However, the lifetime histograms of the sub-

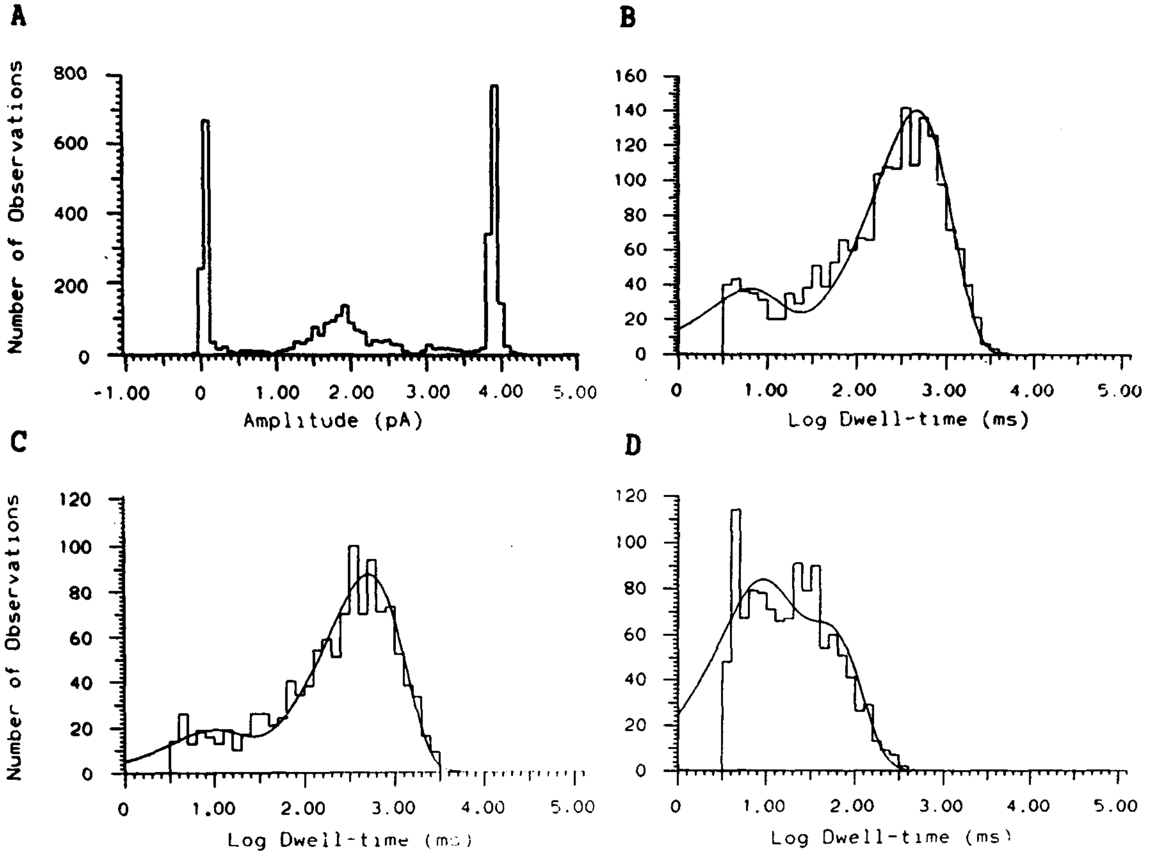

Fig. 9. Dwell time distributions of SRK channels from rat skeletal muscle. Amplitude and dwell time populations were compiled from the data recorded for 30 minutes (1385 events) as described in Fig 4. A, amplitude histogram showing three levels: 0.1, 1.9 and 3.9 pA. B-D, dwell-time histograms of open state (B), closed state (C) and substate (D). Solid lines are fits to the sum of two exponentials. Respective time constants and proportions are: B, 5.63 ms (19%) and 469 ms (81%); C, 7.78 ms (15%) and 519 ms (85%); D, 6.6 ms (48%) and 47 ms (52%).

state were fitted with two time constants (6 and 47 ms) of similar proportions.

K^+ Channels recorded in bilayers formed from extracted membrane lipids

The composition of membrane phospholipids of skeletal muscle cells (Lau et al, 1979) are different from those used for channel incorporation in the usual bilayer method. We extracted the membrane lipid from the microsomes of the rat skeletal muscle. Bilayers fromed from the extracted lipid (25 μg/ml in decane) were good for the incorporation of K^+ channels, but appeared less stable than those formed from pure phospholipids. Fig. 10 illustrates the single channel currents of two K^+ channels of rat skeletal muscle incorporated into a bilayer formed with extracted lipid from the same tissue. Both channels recorded in extracted lipid showed the typical activity of the respective channel type. SRK channels in extracted bilayer were similar in their typical substate behavior and slow gating, but the amplitude of the substate level was

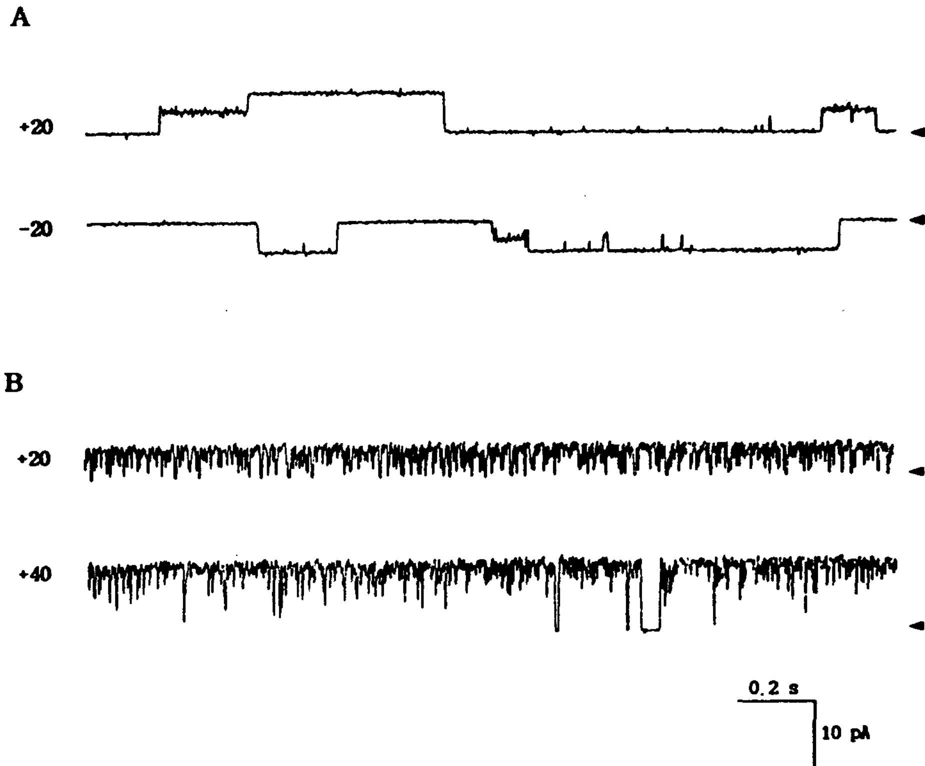

Fig. 10. K^+ channels incorporated into membrane lipids extracted from rat skeletal muscle. Currents were recorded at symmetrical 100 mM KCl and 200 μM $CaCl_2$. Membrane potential across the bilayer is shown on the left and the closed level is indicated by a narrow head on the right. A, current record of an SRK channel with substate at about 60% of open-state level. B, current record of a BK channel incorporated into a bilayer formed with extracted membrane lipid.

about 60% of the open state level (Fig. 10A), indicating that the relative size of substate of the SRK channel recorded in the extracted lipid is larger than that in the pohsphatidylethanolamine/ phosphatidylcholine membrane (0.5, Fig. 9A). It is also interesting that SRK channel were incorporated more frequently into the bilayers formed with the extracted lipids than those with pure synthetic lipids.

DISCUSSION

In the present study, we characterized the basic properties of large Ca^{2+}-activated and sarcoplasmic reticulum K^+ channels from skeletal muscle incorporated into planar lipid bilayers formed from pure and extracted phospholipids.

Large Ca^{2+}-activated K^+ (BK) channel

The unitary properties of BK channels such as conductance, current-voltage relation and sensitivities to voltage and Ca^{2+} were similar in general to those reported in skeletal muscle (Moczydlowski & Latorre, 1983), smooth muscle (Toro et al, 1990; Lee et al, 1991), brain (Reinhart et al, 1989) and renal brush border cells (Zweifach et al, 1991). One obvious difference was that the half-activation voltage, the voltage at which P_o is 0.5, of the BK

channels in this work (16.7± 3.75 mV, n=8) was shifted by about 25 mV compared to the voltage reported by Moczydlowski and Latorre (1983) under similar experimental conditions. It is also clear that our BK channels were less sensitive to Ca^{2+} than those recorded in lipid bilayers from coronary artery (Toro et al, 1990) and renal brush-border membrane (Zweifach et al, 1991). Although the lipid surface charge could cause such a difference (Moczydlowski et al, 1985), one can rule out the surface charge effect because the BK channels in this work and that of Moczydlowski and Latorre (1983) were recorded in a phosphatidylethaolamine bilayer which is electrically neutral. Kapicka et al (1994) showed that BK channels in artificial bilayers required five-fold higher free Ca^{2+} or 80 mV stronger depolarization for activation than BK channels recorded in patches. It is possible that the modification of channel structure, phosphorylation state or regulatory factors during microsomal preparation affected the channel activity (Reinhart et al, 1991; Lee et al, 1994). In addition, such differences could arise from the differences in lipid composition of the two bilayer membranes (Carruthers & Melchior, 1986).

The gating of BK channels showed similar behavior to those of Moczydlowski and Latorre (1983) in that mean open dwell-times are linearly related to Ca^{2+} concentration and mean closed dwell-times are related to the inverse of Ca^{2+} concentration. Based on this and a Hill coefficient that indicates two Ca^{2+}-binding sites, they proposed a reaction model for BK channel gating with two Ca^{2+} binding. However, our data is not compatible with their reaction scheme because the Hill analysis of our data does not indicate more than one Ca^{2+}-binding site.

Although we cannot explain the lower Ca^{2+}-sensitivity and Hill coefficient of the BK channels in the present work, our BK channel should be considered qualitatively identical to the BK channels reported by Moczydlowski and Latorre (1983), Leinhart et al (1989) and Toro et al (1990) in skeletal muscle, brain and coronary smooth muscle because of the qualitative similarities in Ca^{2+}-sensitivity, voltage-dependent gating and their large conductance.

The presence of subtypes of BK channels has been proposed based on the heterogenous slope conductance and gating kinetics in brain and coronary smooth muscle (Reinhart et al, 1989; Toro et al, 1990). The slope conductance of BK channels in this work also showed a considerable variation ranging from 197 pS to 397 pS. It is unlikely that such variation is due to variation in experimental conditions or changes in the lipid environment because BK channels with different conductances were seen in identical recording conditions (this work) as well as in isolated patches (Kapicka et al, 1994). In support of this, Lagrutta et al (1994) reported that alternatively spliced variants of *Slowpoke*, a Ca^{2+}-activated K channel gene (Atkinson et al, 1991) could be distinguished by their unitary conductances, calcium sensitivities and gating kinetics. The question of functional significance of the presence of BK channel subtypes remains to be answered.

Sarcoplasmic reticulum K^+ channel

The substate seen in the second type of channel in this work is a typical feature of SRK channels reported in cardiac muscle (Hill et al, 1990; Uehara et al, 1991). Such a substate cannot be the result of recording two types of channels with different amplitudes because we did not observe a third open-level correspoding to the summed amplitude of substate and open-state at the voltages showing high activity. The substate occurred at 30% of the open-state in frog skeletal muscle but at 50～60% in the rat skeletal muscle (Figs. 6, 9 and 10) and canine cardiac muscle (Hill et al, 1990; Uehara et al, 1991; Shen et al, 1993). In addition, the two

mean closed dwell-times of several (7.8 ms, 15 %) and hundreds of milliseconds (519 ms, 85%) at 20 mV were very similar to those of canine cardiac SRK channels recorded in bilayer (Shen et al, 1993).

Because of their intracellular location, SRK channels have been studied mostly in planar lipid bilayers and the substate phenomena seemed to be a common property of SRK channels (Hill et al, 1990; Uehara et al, this work). However, a recent patch clamp study of the SRK channel in vesicles formed on stretched skeletal muscle fibers revealed a K^+ channel without a substate (Wang & Best, 1994). The weak voltage-dependence and block by decamethonium, a known blocker of SRK channel (Miller, 1982), indicated that the channel shared the properties of SRK channels that exhibited the substate behavior. It remains to be studied further whether the substate phenomena is seen only in channels incorporated into bilayers or whether there are two types of K channels in sarcoplasmic reticulum: one with a substate and the other with no substate. The activity of SRK channels is weakly voltage-dependent with a half-activation voltage of 24 mV and e-fold increase in activity per 35 mV. Similar results were reported in cannine cardiac SRK channels (Shen et al, 1993). In contrast, the half-activation voltage of the SRK channel in isolated patches was about −70 mV (Wang & Best, 1994), indicating a much higher activity of this SRK channel in native membrane.

Physiological roles of BK and SRK channels

Although BK channels are one of the most commonly observed types of ion channels in planar lipid bilayer (Moczydlowski & Latorre, 1983; this work) as well as in the isolated patches (Barrett et al, 1982; Escobar et al, 1993), little has been known about their functional role in skeletal muscle. Based on their Ca^{2+}- and voltage-dependence, one can imagine that they function in re-establishing membrane potential after muscle contraction or a surge of intracellular Ca^{2+} concentration. In metabolically exhausted muscle fibers, BK channels might not play the major role because only a small portion of the K^+ conductance was inhibited by TEA (Castle & Haylett, 1989), a potent blocker of BK channels.

BK channels could be recorded from isolated patches of various smooth muscle cells (Barrett et al, 1982; Lee et al, 1991), hence they were believed to exist on the cell membrane. This is less clear in the mature skeletal muscle. The sarcolemma has been difficult to access directly by patch pipette because of an extensive endomysial network of collagen fibers and a basal lamina on the surface (Ishikawa et al, 1983). The BK channels recorded from isolated patches of cultured myotube (Barrett et al, 1982), may not represent the BK channels of the mature skeletal muscle cells in terms of their level of expression and location. The recent vesicle preparation for patch clamp study developed by Stein and Palade (1989) was initially thought to originate from sarcolemma, and BK channels were routinely observed in the preparation (Escorbar et al, 1993). However, Lewis et al (1992) reported that the preparation also contained proteins, such as ryanodine receptors, which originated from sarcoplasmic reticulum. Further work is required to identify the location of the BK channels in relation to their functional role in skeletal muscle cells.

The contraction of skeletal muscle is triggered by the release of Ca^{2+} from the sarcoplasmic reticulum (SR) into the myoplasm. It is known that the SRK channels mediate a shunt conductance across the SR membrane and compensate for the charge movement caused by Ca^{2+} efflux via ryanodine-sensitive Ca^{2+}-release channels during excitation-contraction coupling (Caille et al, 1985; Abramcheck & Best, 1989). One mechanism that operates SRK channels could be deduced from

their voltage dependence. The voltage across SR membrane is probably 0 mV in resting state because the ionic composition of myoplasm and SR are homogeneous except for Ca^{2+} (Somlyo et al, 1977). However, rapid efflux of Ca^{2+} during activation might polarize the SR membrane causing a transient change in voltage that might regulate the activity of SRK channel.

Blockers of SRK channels such as BisG10 decrease the amount of Ca^{2+} released from the SR (Abramcheck & Best, 1989). One might expect that any endogenous substance that can block SRK channels reduces the contractile force. Especially, the changes in metabolites such as ATP, phosphocreatine, lactate, H^+ and NADH during muscle fatigue or ischemia are well established (Green et al, 1992; Nagesser et al, 1992). Therefore, it is highly possible that one or more of these endogenous substances regulate the activity of SRK channels in the resting or fatigue states of skeletal muscle. For example, H^+ decreased the conductance of the SRK channel of skeletal muscle (Bell, 1985) and Ca^{2+} activated the cardiac SRK channels (Uehara et al, 1991).

Membrane phospholipid and ion channel activity

In forming planar lipid bilayer phosphatidylethanolamine (PE), we usually use pure phosphatidylethanolamine (PE), or combination of PE with phosphatidylcholine (PC) and/or phosphatidylserine (PS) such as PE (100%), PE/PC (70/30%) or PE/PS (60/40%) to achieve an adequate rate of fusion. However, the actual membranes have a quite different lipid composition. According to Lau et al (1979), sarcoplasmic reticulum membranes were composed of PC (68%), PE (17%), phosphatidylinositol (8%), PS (2%), and sphingomyelin (4%) but T-tubules were composed of PC (45%), PE (26%), phosphatidylinositol (4%), PS (8%) and sphingomyelin (16%). In addition, T-tubule membranes had four times more cholesterol than SR membranes. The major difference between the native membranes and the lipid composition of the bilayer used for this work is in the amount of PC (less than 30% of total phospholipid) and cholesterol (no cholesterol added). Although it was not the aim of the present work to study the lipid-channel interaction, the differences of substate level and fusion rate of SRK channels in extracted lipid suggest that membrane lipid could be an important regulatory factor of ion channel activity in normal as well as pathological states.

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