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Characterization of the potassium channel from frog skeletal muscle sarcoplasmic reticulum membrane

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1. The sarcoplasmic reticulum (SR) membrane of skeletal muscle contains potassium channels which are thought to support charge neutralization during calcium release by providing a permeability pathway for counter-ion movement. To describe the behaviour of the SR K^+ channel under physiological conditions, single channel activity was recorded from excised patches of SR membrane. Patches were made from membrane blebs extruded from contracted muscle fibres whose surface membranes had been removed previously by mechanical dissection.
2. The channel was active over a large voltage range from −80 to +100 mV. The current–voltage relationship of the channel was linear over most of this voltage range (slope conductance equal to 60 pS in 130 mM potassium), but showed rectification at voltages below −50 mV.
3. The activity of the channel (number of state transitions per unit time) was greater at positive voltages than at negative voltages. Analysis of dwell-time distributions showed that the time spent in the open state is best fitted by a double Gaussian, suggesting that the channel possesses both a long (l)- and a short (s)-lived open state with identical conductances. The dwell times for the two states were $T_s = 0{\cdot}3$ ms and $T_l = 2{\cdot}6$ ms at +90 mV and $T_s = 0{\cdot}1$ ms and $T_l = 15{\cdot}1$ ms at −40 mV. Thus, positive voltage decreased the long open time significantly which was consistent with the observed increase in channel activity at positive potentials.
4. The permeability sequence of the channel to various monovalent cations was deduced from the channel reversal potential under bi-ionic conditions and was found to be: $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$.
5. Channel activity was reduced when the patch was perfused with 1,10-bis-guanidino-*n*-decane (BisG10), a drug reported to block the SR K^+ channel with high affinity. The drug concentration necessary to reduce the open probability (P_o) by 50% was 19·8 μM at −40 mV and 338·2 μM at +50 mV. The zero voltage dissociation constant (K_d) was calculated to be 48 μM.
6. Pharmacological agents known to affect surface membrane K^+ channels, such as 0·5 mM Ba^{2+} or 3·0 mM 4-aminopyridine, were much less effective in blocking the channel than BisG10. Physiological calcium concentrations (pCa = 8·0 and 3·0) did not affect channel behaviour.
7. The high open probability at 0 mV ($P_o = 0{\cdot}9$) and relative insensitivity of P_o to voltage and calcium are consistent with the idea that the SR K^+ channel is a major pathway for counter-ion movement during Ca^{2+} release.

During skeletal muscle activation, an intracellular organelle known as the sarcoplasmic reticulum (SR) releases Ca^{2+} into the myoplasm to cause contraction. In addition to the Ca^{2+} release channel, the SR membrane is known to contain ion channels selective for monovalent cations and anions (Meissner, 1983; Miller, Bell & Garcia, 1984; Hals, Stein & Palade, 1989). These channels are thought to allow the movement of counter-ions across the SR membrane during Ca^{2+} release thus minimizing charge separation and the development of a membrane potential that would retard continued Ca^{2+} efflux (Miller, 1978; Oetliker, 1982). Thus, the existence of permeability pathways for counter-ion movement is thought to be essential for maintaining the large Ca^{2+} flux that occurs during muscle activation.

This interpretation is supported by the observation that decreasing monovalent cation permeability decreases the rate of Ca^{2+} release from the SR in single muscle fibres (Abramcheck & Best, 1989).

Given the essential physiological role played by counterions in the normal functioning of the SR, it is of some interest to understand the mechanisms that allow counterion movement. It has been shown that the SR K^+ permeability results from a K^+-selective ion channel whose behaviour has been investigated by reconstituting channel-containing vesicles or purified channels into planar lipid bilayers (McKinley & Miller, 1977; McKinley & Meissner, 1978; Kometani & Kasai, 1978; Coronado, Rosenberg & Miller, 1980; Tomlins & Williams, 1986; Ide, Morita, Kawasaki, Taguchi & Kasai, 1991) and solubilized proteins into liposomes (Tomlins & Williams, 1986). Under these conditions the channel is weakly voltage dependent, is marginally selective for K^+ over other monovalent ions and is sensitive to block by quaternary amines. While the behaviour of the SR K^+ channel in artificial bilayers has been extensively described, little information is available concerning the behaviour of the channel *in situ*. Direct access to native SR membrane is possible using split lobster muscle (Tang, Wang & Eisenberg, 1989) and this preparation has provided information about SR K^+ channel function in crustaceans. However, many of the physiological studies related to calcium release during excitation–contraction have used frog skeletal muscle. An understanding of the *in situ* behaviour of the K^+ channel from frog SR is important if the mechanisms of charge redistribution during excitation–contraction coupling are to be understood.

In this paper we present experiments using a preparation first described by Stein & Palade (1988) that allows patch clamp studies of frog SR membrane. Our primary goal was to describe the conductance and gating behaviour of the SR K^+ channel in a lipid and ionic environment close to that which occurs physiologically and to compare this behaviour to that of channels reconstituted into artificial membrane systems.

METHODS

Membrane preparation

SR membrane was extruded from single muscle fibres whose surface membranes had been removed previously by mechanical dissection (Stein & Palade, 1988). Frogs (*Rana pipiens berlandieri*) were killed by decapitation and then pithing. The semitendinosus muscle was isolated and placed in cold Ringer solution. A small bundle of muscle fibres was cut from the semitendinosus muscle, blotted dry and put into a relaxing solution containing (mM): 130 potassium glutamate, 2 EGTA (pCa = 8·0), 1 MgATP, 2 free Mg^{2+}, and 15 Hepes (pH = 7·0). A single fibre was then separated from the bundle and one end of the fibre was attached to a small clip made of aluminum foil that was then mounted on a hook glued to the bottom of the recording chamber. Starting from the free end, the sarcolemmal membrane of the single muscle fibre was removed by mechanical dissection. The 'skinned' fibre was then contracted by changing the bath solution to one containing 130 mM potassium glutamate and with a pCa value of 3·0. During the contraction, membrane was extruded from the fibre interior to the surface where hemispherical blebs of membrane became visible. The diameter of the blebs varied from 50 to 150 μm and they were easily contacted by patch pipettes. The rationale for assuming that the membrane that formed these blebs was predominately of SR origin is given in the Discussion.

Recording solutions

The composition of the recording solutions was chosen to mimic physiological conditions assuming the cytoplasmic side of the SR membrane faced the pipette interior. Pipette solutions were calculated to contain (mM): 130 K^+, 2 EGTA (pCa = 8·0), 1 MgATP, 1 free Mg^{2+} and 15 Hepes (pH = 7·0). The bath solution contained (mM): 130 monovalent cation (K^+, Cs^+, Li^+, Rb^+ or Na^+), 1 Ca^{2+} (pCa = 3·0), 1 Mg^{2+}, and 3 Hepes (pH = 7·0). Glutamate was used as the major anion in all solutions. In addition, pipette solutions contained about 4 mM Cl^- and bath solutions 2 mM Cl^- added as the magnesium salt.

Electrophysiology

Electrodes were pulled from hard glass capillary tubes (Corning 7052) by a two-stage puller (Model PP–83, Narishige, Japan), coated with Sylgard (Dow Chemical Corporation) and fire-polished to have a tip resistance between 20 and 40 MΩ when filled with pipette solution.

Once the pipette contacted the membrane bleb, gigaohm seals were easily obtained by releasing the slight positive pressure that had been applied to the patch pipette before it was placed in the bath. Suction was not used to form seals. With low resistance electrodes the entire bleb was pulled into the pipette under negative pressure. With the high resistance pipettes used in this study, seal formation was adequate without the use of negative pressure. Once a seal was made, the electrode was removed from the bleb to form an excised patch with the cytoplasmic side of the SR membrane facing the pipette interior and the lumenal side of the SR membrane exposed to the bath solution (Stein & Palade, 1988). Single channel data were recorded from excised inside-out patches with seal resistances greater than 10 GΩ. An Axopatch 1B patch clamp (Axon Instruments, Inc.) interfaced to a personal computer was used for voltage control and data acquisition. Voltages are reported as pipette potentials referenced to the earthed bath. The majority of the data were filtered at 5 kHz with an eight-pole Bessel filter before being sampled at 10 kHz. For some experiments sample rates as high as 50 kHz were used with the filter adjusted accordingly. The experiments were performed at room temperature (about 20 °C).

Single channel analysis was performed with commercially available software that utilized a half-maximum threshold detection criterion (pCLAMP, Axon Instruments, Inc.). Data were recorded after stepping the patch from a holding potential of 0 mV to various test potentials.

Chemicals

All chemicals used were reagent grade. 4-Aminopyridine was purchased from Sigma. BisG10 (1,10-bis-guanidino-*n*-decane) was synthesized following the method of Garcia & Miller (1984*a*).

RESULTS

In addition to potassium channels, the SR membrane is known to contain Ca^{2+} and Cl^- channels (Smith, Imagawa, Ma, Fill, Campbell & Coronado, 1988; Hals *et al.* 1989). We expected that many excised patches would contain more than one type of ion channel thus complicating analysis of K^+ channel behaviour. In practice, the presence of Cl^- channels in a patch did not present significant difficulties. Block of current through Cl^- channels was easily accomplished by using the impermeant anion glutamate as a substitute for most of the Cl^- in the bathing solutions (Hals *et al.* 1989). The presence of SR Ca^{2+} channels was more problematic since this channel is not highly selective for divalent over monovalent ions and, in fact, conducts K^+ rather well (Smith *et al.* 1988). Therefore, we relied on differences in the voltage dependency and selectivity of the K^+ and Ca^{2+} channels as diagnostic criteria to differentiate between these types of channels. Activity of the SR Ca^{2+} channel in extruded SR membrane is limited to a rather narrow voltage range (from about -20 to about $+30$ mV; Stein & Palade, 1988; Kwok & Best, 1990), while the SR K^+ channel is active over a much wider voltage range (from -80 to $+100$ mV). In addition, the SR K^+ channel is poorly conductive for Cs^+ (Cukierman, Yellen & Miller, 1985; Abramcheck & Best, 1989) whereas the SR Ca^{2+} channel is highly conductive for this ion (Smith *et al.* 1988).

Typical current responses obtained from two patches, one containing a Ca^{2+} channel and the other a K^+ channel, are shown in Fig. 1. The stimulus was a voltage ramp from -50 to $+50$ mV as shown diagrammatically at the top of the figure. Bath and pipette solutions contained equimolar K^+ except for the bottom trace where the potassium in the bath had been replaced by Cs^+. The continuous line in each current trace corresponds to the level of the leakage current that remains when no channels are open in the patch. The seal resistance is equal to the slope of this line and was greater than 10 GΩ for the traces shown. Characteristically, the Ca^{2+} channel is open over a relatively narrow voltage range as compared with the K^+ channel. Although the calcium channel opens immediately in response to the initial voltage change from 0 to -50 mV, it closes again within the first few hundred milliseconds and does not reopen until a voltage of about -20 mV is reached. The open probability then remains high until about $+30$ mV when increased numbers of closures are seen. In contrast, the SR K^+ channel has a high open probability throughout the entire ramp. The identity of the K^+ channel was confirmed by replacing the normal bath solution with one containing Cs^+, rather than K^+, as the major monovalent cation. With Cs^+ in the bath, the conductance of the K^+ channel dropped markedly when the driving force for monovalent cations was directed from the bath into the pipette.

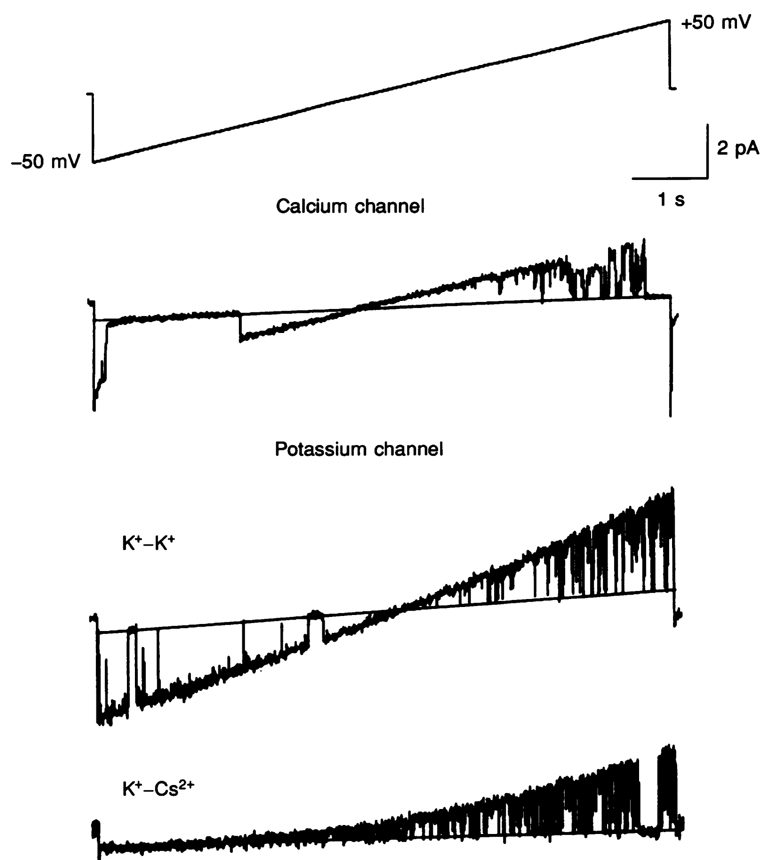

Figure 1. Identification of Ca^{2+} and K^+ channels in extruded SR membrane with monovalent cations as charge carriers
Patches were stimulated by a voltage ramp (13 mV s^{-1}) from -50 to $+50$ mV as shown schematically at the top. Both pipette and bath solutions contained 130 mM potassium except as noted below for the third data trace. The first data trace shows the behaviour of a patch containing a Ca^{2+} channel. Note that the channel opens over a limited voltage range (about -20 to $+30$ mV). The second and third data traces illustrate the behaviour of patches containing single K^+ channels. Channel activity is high throughout the full voltage range. In the third data trace, the bath contained Cs^+ ions which are relatively impermeable through the SR K^+ channel. Note the decrease in conductance at negative voltages when Cs^+ is the major charge carrier. The different voltage dependency and ion selectivity of the channels allowed them to be easily differentiated.

We recorded data from 721 patches, and found that 284 of them showed no channel activity, 146 contained both K^+ and Ca^{2+} channels, 147 contained only Ca^{2+} channels, and 144 contained only K^+ channels. Since Cl^- channels are electrically silent in the recording solutions we used, we do not know how many patches might have contained Cl^- channels. For this report we analysed data from a portion of the 144 patches containing only K^+ channels.

Voltage dependency and conductance of the potassium channel from extruded SR membrane

Typical single K^+ channel current traces recorded from excised patches bathed in symmetrical 130 mM K^+ solutions are shown in the upper portions of Fig. 2*A* and *B*. Currents were recorded after the patch was stepped from a holding potential of 0 mV to the test potential indicated. Amplitude histograms describing channel activity when the patches were held at 100 mV are shown as inserts in the *I–V* plots. Only a single open current level is distinguishable in the records shown in Fig. 2*A*. A single open current level was found in 31 of the 144 patches studied. The summed duration of stored data traces obtained for most of these patches ranged from 10 to 15 s although in one experiment we stored several minutes of data. No evidence of a second open current level was ever seen at any test potential in these 31 patches. In contrast, 48 patches had channel activity that showed two levels of open channel current. An example of this type of behaviour is seen in Fig. 2*B*. The remaining 65 patches containing three or more open current levels were not analysed further.

Average current–voltage relationships are shown below each set of current traces. They were constructed from pooled data taken from the thirty-one single open current level patches and the forty-eight two open current level patches. The *I–V* curves are roughly linear at positive potentials, but showed significant rectification at negative

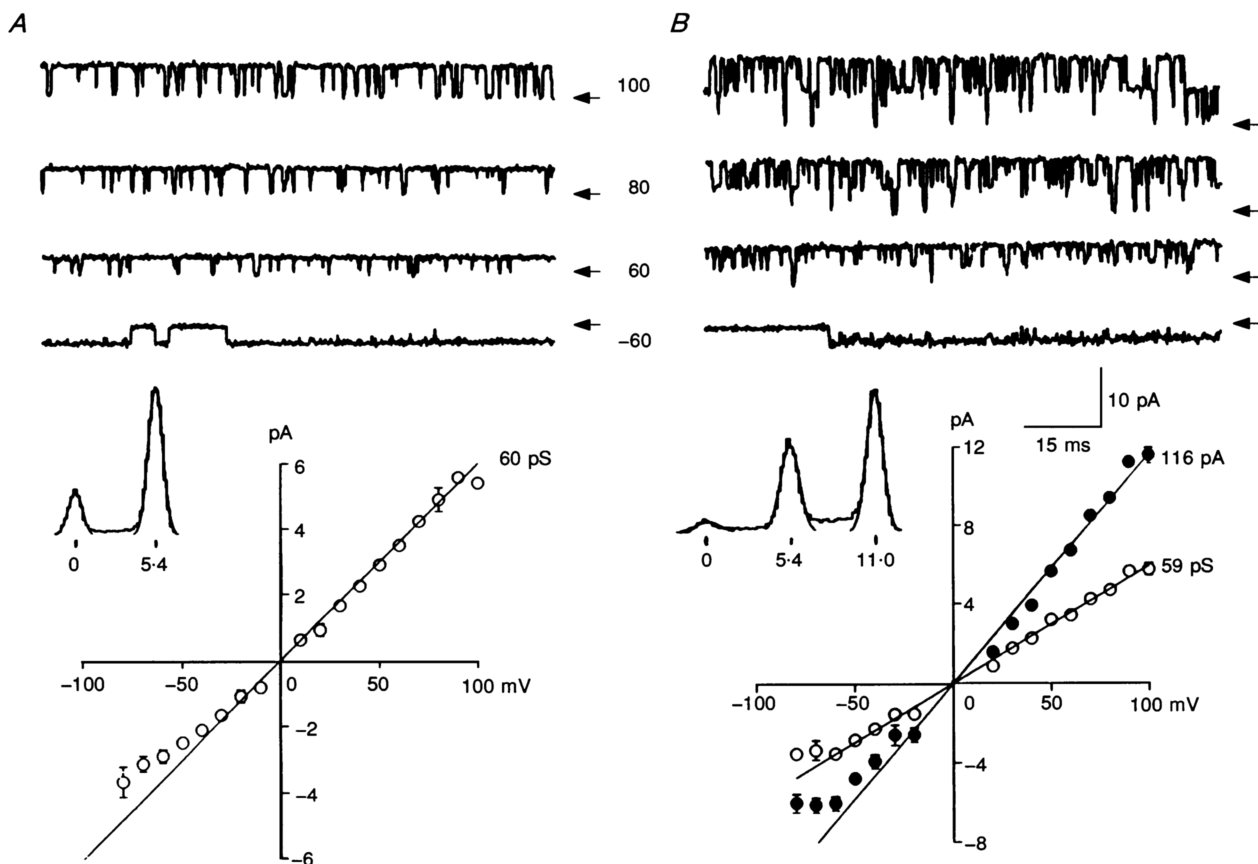

Figure 2. Typical single channel records from a patch that showed only one open current level of 60 pS (*A*) and a patch having two, electrically distinct, current levels of 59 and 116 pS (*B*)
Solutions contained 130 mM K^+. Patches were held at 0 mV before being stepped to the voltages indicated. Arrows indicate zero current level. Typical amplitude histograms (for currents measured at +100 mV) are shown below the data traces for these two patches. Current–voltage relationships give the averaged response (means ± S.E.M.) from 31 single current level patches and 48 patches with two open current levels.

voltages. The amount of rectification that occurred at negative voltages varied somewhat between different channels but was always present. We estimated the slope conductance by fitting a line to the current values obtained at positive potentials. For the single current level patches, the average slope conductance was 60 pS. The average conductances for the two open current level patches were 59 and 116 pS. An obvious aspect of the behaviour of the K^+ channels shown in Fig. 2 is that channel gating, as manifested by both mean open probability and number of state transitions per unit time, is voltage dependent. The dependence of mean channel open probability on voltage is shown in Fig. 3. The values of P_o are averages calculated from all the current records recorded from the thirty-one single conductance level patches and the forty-four two conductance level patches. In general, the channel can be described as being weakly voltage sensitive; the open probability is high except at large negative potentials. Panel *A* describes the voltage dependency of P_o for patches having only one open channel current level corresponding to a conductance of 60 pS. The average P_o was approximately 0·9 for voltages between −60 and +40 mV. P_o decreased sharply at voltages below −60 mV and a smaller, and more gradual drop in average P_o occurred at voltages above 40 mV. The voltage dependency of P_o for patches containing two open channel current levels is seen in panel *B*. The P_o for the 120 pS state ranges from 0·6 to 0·8 for voltages from −60 to +50 mV. The P_o for the 60 pS state varies from 0·2 to 0·35 over the same voltage range.

Previous descriptions of the SR K^+ channel studied in artificial bilayers describe the channel as having both a large conductance state and a less often observed subconductance state (Labarca & Miller, 1981; Fox, 1985, 1987). We have never observed a subconductance state that we feel is consistent with these earlier observations. Consideration of the behaviour of the patches with two open current levels as described above leads us to believe that they contain two ion channels and not a single channel with two different conductance states. Arguments in support of this conclusion are offered in the Discussion. The rest of this paper describes experiments performed on patches with only a single open current level which we assume are patches containing a single ion channel.

Voltage dependency of channel kinetics

As mentioned previously, channel activity as indicated by the number of state transitions per unit time is greatly increased at large positive potentials as compared with large negative potentials (Fig. 2). To investigate the mechanism underlying this change, we used dwell-time analysis to determine the average time spent by the channel in open and closed states. A histogram with a logarithmic time axis and a square-root vertical axis was used to display the distributions of dwell times from recordings of SR K^+ channels (Sigworth & Sine, 1987). The top trace of Fig. 4*A* shows typical single channel currents recorded at a holding potential of +90 mV. The histogram in Fig. 4*A* shows the dwell-time distribution of the open channel at 90 mV. Data from eight patches showing only the 60 pS state were pooled together to increase the total recording time to 16 s and total number of events to 11097 for the open dwell-time analysis. The two peaks indicate two distinct mean open times for the channel, one short ($T_s = 0·3$ ms) and one long ($T_l = 2·6$ ms). Since we could

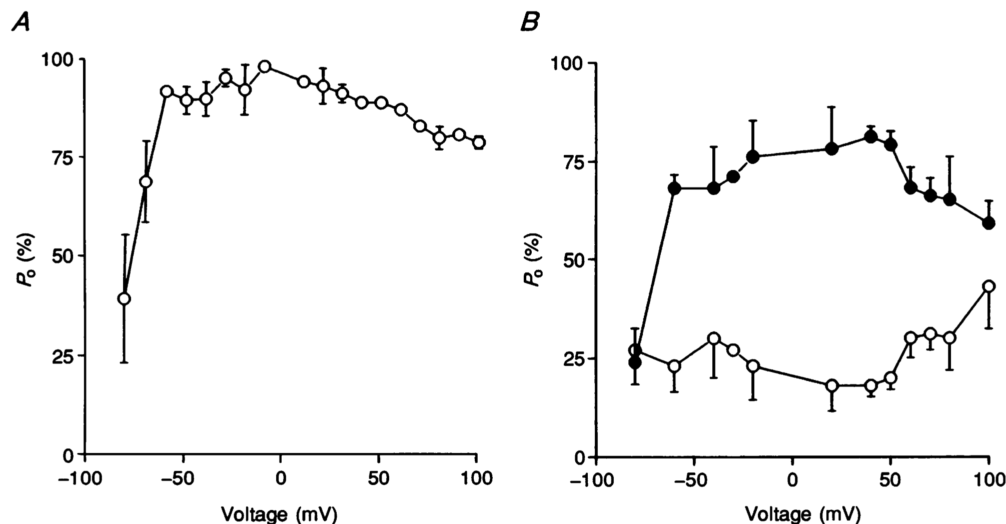

Figure 3. Average voltage dependency of channel open probability (means ± S.E.M.)
Data taken from the 31 patches having a single current level (*A*) and 48 having two open current levels (*B*). In *B* the open and filled symbols refer to the low and high conductance states, respectively.

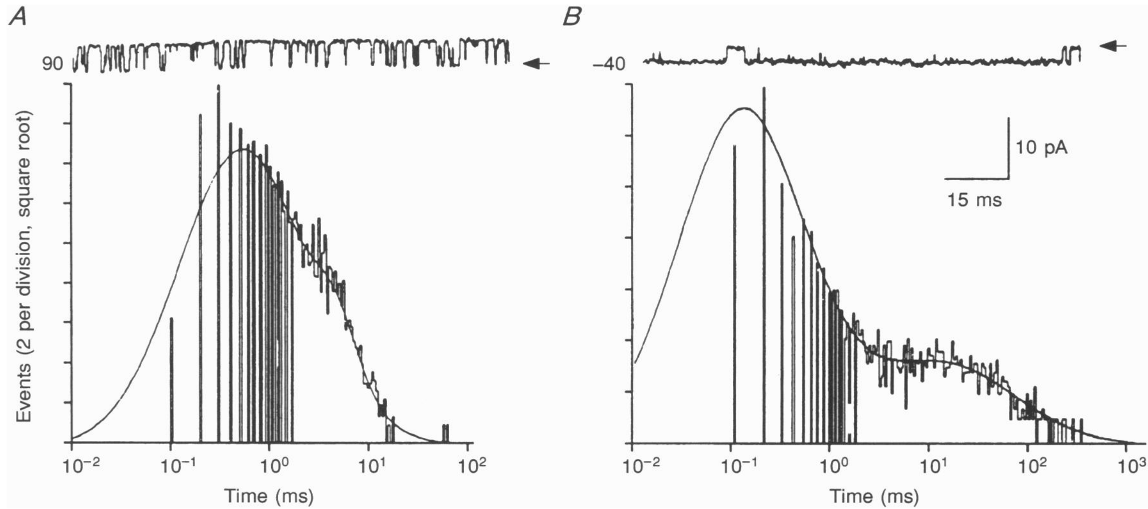

Figure 4. Dwell-time analysis of currents from patches having a single open state
Data trace is a sample of single channel current used for analysis. Arrow indicates the zero current level. Test potential is 90 mV. *A*, the dwell-time histogram indicates the existence of two mean open times of 0·3 and 2·6 ms. *B*, as in panel *A* but $V_h = -40$ mV. The mean open times are 0·1 and 15·1 ms. Single channel data from eight patches were pooled together to increase the number of events analysed to 11097 at 90 mV and 3553 at −40 mV.

identify only one open current level in the data traces, the two open states are presumed to have identical conductnces. Dwell-time analysis was also performed on data from patches held at −40 mV (Fig. 4*B*). The total number of events analysed in this case was 3553. Two mean open times were again present ($T_s = 0·1$ and $T_l = 15·1$ ms). The mean duration of the long open state is increased at −40 mV as compared with that seen at +90 mV. This result is consistent with the appearance of long periods of open channel current in the single channel records seen at −40 mV.

A similar dwell-time analysis was attempted for the closed states. Qualitatively the data suggest the existence of two mean closed times. However, due to the small number of long closed events and the variability in their duration, particularly at −40 mV, reasonable Gaussian fits to the data were not obtained. We felt the time constants generated by the analysis to be unreliable and have therefore not included them in this report.

Selectivity as determined by reversal potential

The selectivity of the SR K^+ channel was determined by measuring the zero current (reversal) potential under bi-ionic conditions. The internal solution always contained 130 mM K^+, while the bath solution contained 130 mM of one of the following monovalent cations: Rb^+, Li^+, Cs^+ or Na^+. Representative current traces and the resultant *I–V*

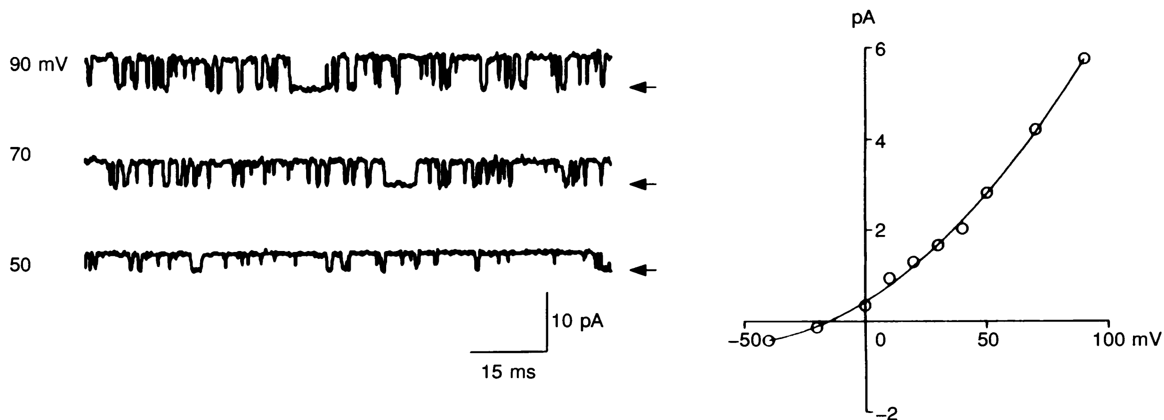

Figure 5. Channel selectivity as determined by reversal potential under bi-ionic conditions
The pipette solution contained 130 mM K^+, and the bath solution contained 130 mM Rb^+. The data were fitted as described in the text. The calculated P_K/P_{Rb} was 1·8.

Table 1. Permeability ratios of various cations

Cation (X)	P_K/P_X *
Rb^+	1·7 ± 0·1 (4)
Na^+	2·2 ± 0·1 (4)
Cs^+	2·7 ± 0·2 (6)
Li^+	3·3 ± 0·4 (7)

* Means ± S.E.M. with the number of values in parentheses.

curve for a patch containing a single current level are shown in Fig. 5. Current was recorded with 130 mM K^+ in the pipette and 130 mM Rb^+ in the bath solution. The data were fitted to a second-order polynomial and the zero current potential determined to be −15·2 mV yielding a calculated permeability ratio (K^+ *versus* Rb^+) of 1·8. Reversal potentials were determined in this way for each of the cations studied and used to calculate the permeability ratios shown in Table 1. The channel is only slightly selective amongst the monovalent cations studied and has a permeability sequence of $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$.

The channel is not regulated by calcium

A series of experiments were performed to determine whether the channel was sensitive to changes in Ca^{2+} concentration in a range consistent with physiological fluctuations of Ca^{2+} during muscle activation. The bath concentration of Ca^{2+} was kept at 1 mM to correspond to the ionized calcium level in the SR lumen while the pipette Ca^{2+} was set at either pCa = 8·0 or 3·0 to correspond to resting and saturating cytoplasmic Ca^{2+} concentrations, respectively. Current–voltage relationships obtained at different Ca^{2+} concentrations were indistinguishable (data not shown). Also, we did not detect any significant differences in open probability or gating behaviour of the K^+ channel at any of the calcium concentrations studied.

Pharmacological characterization

Block of the channel by pharmacological agents was investigated to allow comparison with K^+ channels from other preparations (Fig. 6). Amplitude histograms were constructed both before and after addition of the test substance to the bath. Typical control current traces elicited at +70 mV and the amplitude histogram generated from them are shown in Fig. 6*A*. The histogram shows a large peak at 3·9 pA and a smaller peak centred at 0 pA that correspond to the open and closed states, respectively. The data in panel *A* are typical of the control current traces of all the patches studied. Neither 3 mM 4-aminopyridine nor 0·5 mM $BaCl_2$ blocked the channel when added to the bath (Fig. 6*B* and *C*). The amplitude histograms generated in the presence of these agents are essentially identical to the control. Both 4-AP and Ba^{2+} are specific blockers of plasmalemmal K^+ channels (Rudy, 1988). Addition of 1 mM

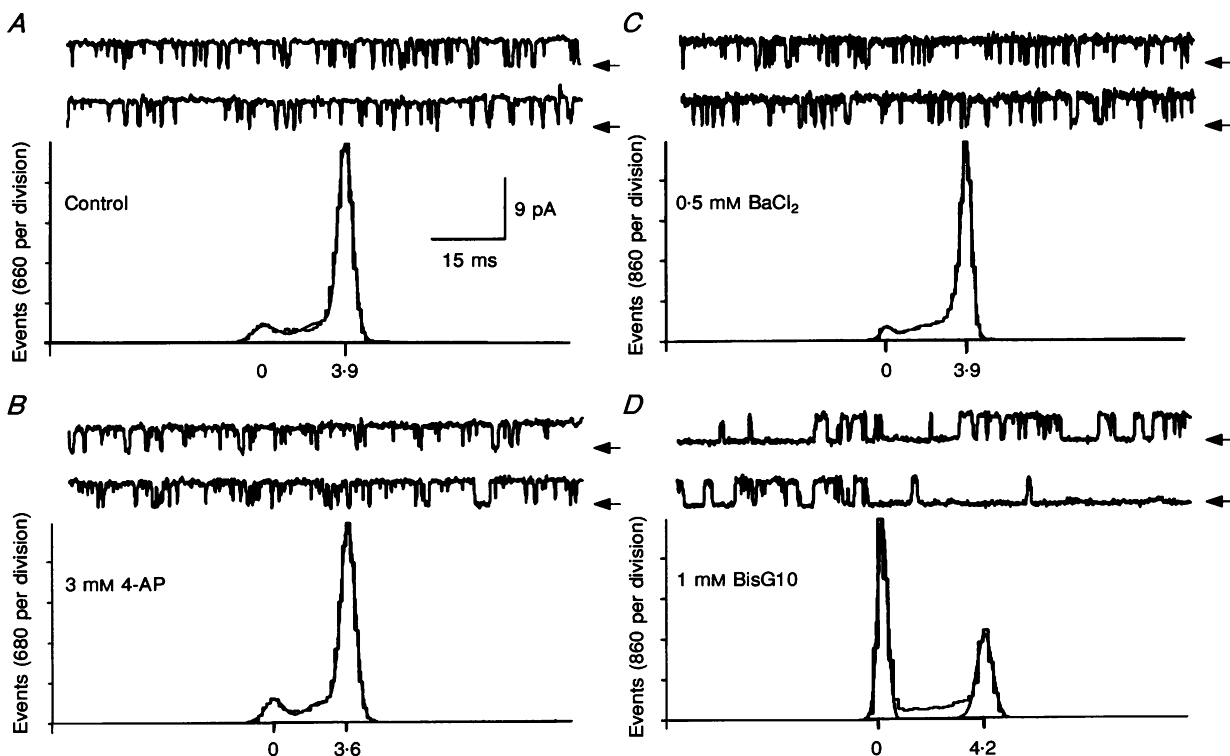

Figure 6. Pharmacological characterization of the SR K^+ channel

Comparison of the behaviour of a channel in symmetrical 130 mM K^+ solution under control conditions (*A*) and in the presence of 3 mM 4-aminopyridine (*B*), 0·5 mM $BaCl_2$ (*C*), and 1 mM bis-guanidino-*n*-decane (*D*). Note blocking effect of bis-guanidino-*n*-decane.

1,10-bis-guanidino-*n*-decane (BisG10) to the bath solution reduced channel open probability (Fig. 6*D*) as indicated by the increase in the area under the peak centred at 0 pA. BisG10 is a specific SR K^+ channel blocker with higher affinity for the channel than the more commonly known quaternary ammonium compounds such as decamethonium (Miller, 1982; Garcia & Miller, 1984*a*, *b*)

The voltage and concentration dependency of BisG10 block are shown in Fig. 7. The current traces in Fig. 7*A* were recorded in bathing solutions containing 1–400 μM BisG10 at a holding potential (V_h) of −40 mV. At this voltage, the drug-free channel remains in the open state for long periods with no closures. As the concentration of the drug is increased interruptions of the long-lived open states become more frequent. At very high drug concentrations, the 'flicker' caused by drug binding and unbinding becomes so rapid that the open current levels are no longer fully resolved. This flickering is reminiscent of the behaviour of the quaternary ammonium blockers with carbon chain lengths greater than nine (Miller, 1982). The relationship between the fractional reduction of channel P_o and blocker concentration is shown in Fig. 7*B* at two holding potentials (−40 and +50 mV). The Hill equation ($P_{inhib} = [\text{BisG10}]^n / \{[\text{BisG10}]^n + Q\}$) was used to fit the data giving values of $n = 1{\cdot}11$, $Q = 27{\cdot}5$ μM at $V_h = -40$ mV and $n = 0{\cdot}97$, $Q = 284$ μM at $V_h = +50$ mV; where P_{inhib} is the fractional inhibition, n is the slope at 50% inhibition and Q is a constant. These constants were used to calculate the concentration of BisG10 that could reduce the P_o by 50%, which was 19·8 μM at $V_h = -40$ mV and 338·2 μM at $V_h = +50$ mV. Thus negative potentials inside the pipette enhance the effect of this positively charged drug.

Further information concerning the interaction of BisG10 and the channel can be gained from analysis of the effect of holding potential on the degree of channel block as shown in Fig. 8 (Miller, 1982). The data relate the extent of block as a function of voltage in the presence of 0·025 mM drug and are fitted to the following equation (Tomlins & Williams, 1986):

$$\ln(G_c/G_b - 1) = \ln([B]/K_{b(0)}) + zdVF/RT,$$

where G_c is the control conductance, G_b is the conductance in the presence of drug, $K_{b(0)}$ is the dissociation constant at zero voltage, z is the valency of the blocker, $[B]$ is drug concentration and d is the fraction of the voltage drop across the membrane at the site of the block. The values of zd (effective valence) and K_b calculated from the data in Fig. 8 are −0·76 and 0·048 mM, respectively.

DISCUSSION

The aim of this work was to characterize the behaviour of the SR K^+ channel in its 'native' membrane under ionic conditions close to those that occur physiologically. While much of the channel's behaviour is similar to that reported from earlier studies of membrane vesicles reconstituted into artificial bilayers, there are some significant differences.

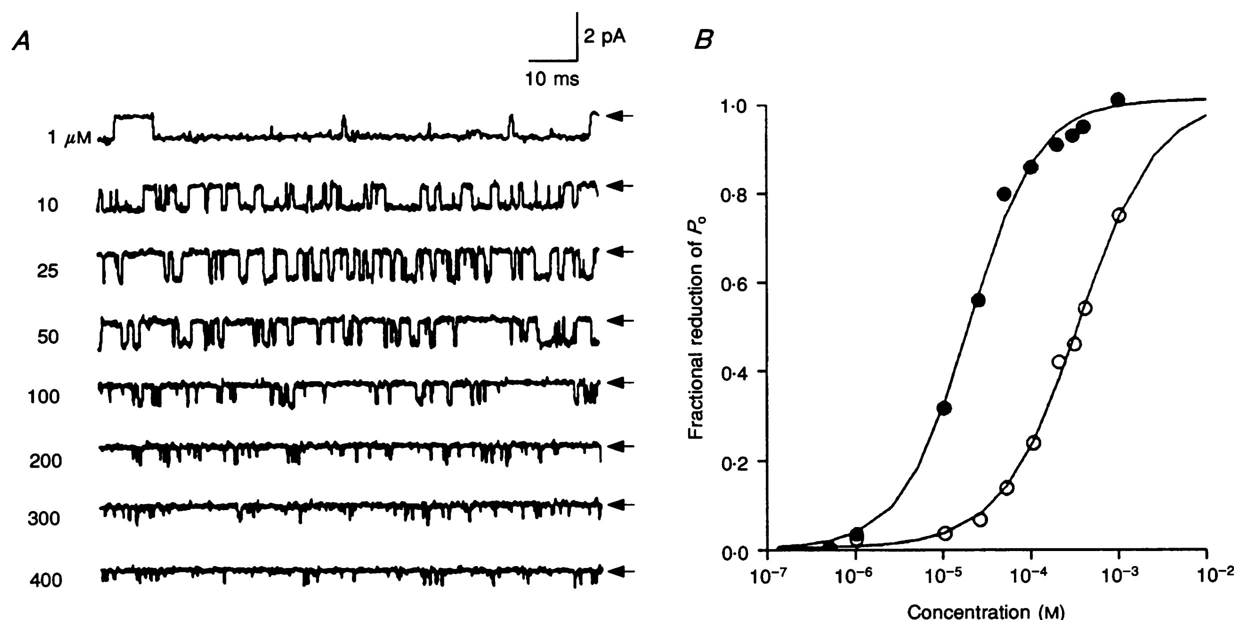

Figure 7. Block by bis-guanidino-*n*-decane (BisG10)
A, single channel current was recorded with different amounts of BisG10 added to the bath solution at $V_h = -40$ mV. Both the P_o and the apparent conductance of the channel were decreased as the concentration of BisG10 was increased. *B*, dose–response curve for BisG10 at two holding potentials (−40 mV, ●; + 50 mV, ○).

How many conductance levels?

A consistent finding in previous investigations of SR K^+ channel behaviour is the existence of two, electrically distinct, open channel conducting states. The larger state is reported to have a conductance of 100–150 pS in K^+ solutions near 100 mM (e.g. Labarca & Miller, 1981; Ide *et al.* 1991; Hirashima, Ishibashi & Kirino, 1991). A substate having a conductance of 60–80 % of the parent state has been reported in SR K^+ channels from frog and crustacean skeletal, as well as rabbit and canine cardiac and skeletal, muscle (Larbarca & Miller, 1981; Tomlins, Williams & Montgomery, 1984; Gray, Montgomery & Williams, 1985; Fox, 1985, 1987; Hill, Coronado & Strauss, 1989; Tang *et al.* 1989). The subconductance state is typically rare, appearing for less than 20 % of the channel open time and has identical selectivity and monovalent ion binding affinity as the parent state (Fox, 1985).

Comparison of the behaviour of patches with either one or two open current levels as described in this report suggests that the SR K^+ channel recorded from extruded SR membrane has only one electrically identifiable conductance state. We base this conclusion on the following observations. First, in thirty-one patches we found only one open current level. The data traces from these patches contain over 10000 state transitions recorded at many voltages. A subconductance state, if it existed, would have to be exceedingly rare for us not to have seen it. Second, in patches in which two open current levels were seen (forty-eight patches) the larger was always twice the conductance of the smaller. This is unlike the subconductance states described previously which were typically 60–80 % of the parent state but is what one would expect from a patch containing two identical channels each with a single conductance state. In fact, for patches with two open current levels the frequency with which the closed and open levels appeared could be predicted theoretically assuming two independent channels. To illustrate this point, we will consider the behaviour of a patch held at −30 mV. For a single open current level patch, the average P_o at this voltage is 0·9 (see Fig. 3*A*). We assume a patch contains two independent channels both with an open probability (p) of 0·9 and a closed probability (q) of 0·1. The probability of both being in the open state at the same time is 0·81 ($p_1 \times p_2$), of only one being open is 0·18 ($p_1 \times q_2 + p_2 \times q_1$), and of neither being open is 0·02 ($q_1 \times q_2$). The actual average probabilities calculated from pooled data taken from six patches at this voltage were 0·71, 0·27 and 0·02, which agrees reasonably well with the predicted values for a two-channel patch. Thus, our data provide no support for the existence of an electrically distinct subconductance state.

Considering the number of single channel events we have analysed it seems unlikely that we would have failed to see a substate if one existed. What then accounts for this discrepancy between the behaviour of the channel in extruded SR membrane and that in channels reconstituted into bilayers? The dwell-time analysis described in Fig. 4 indicates the existence of two, electrically indistinct open states for the channel we have studied. Perhaps the isolation procedures or different lipid environments used in other studies alters channel function so that the conductances of these two states are not identical and thus are electrically distinguishable. An effect of lipid composition on SR K^+ channel function is well documented. Bell & Miller (1984) have shown that the conductance of rabbit SR K^+ channels is affected by the surface potential of the membrane due to alterations in the concentration of K^+

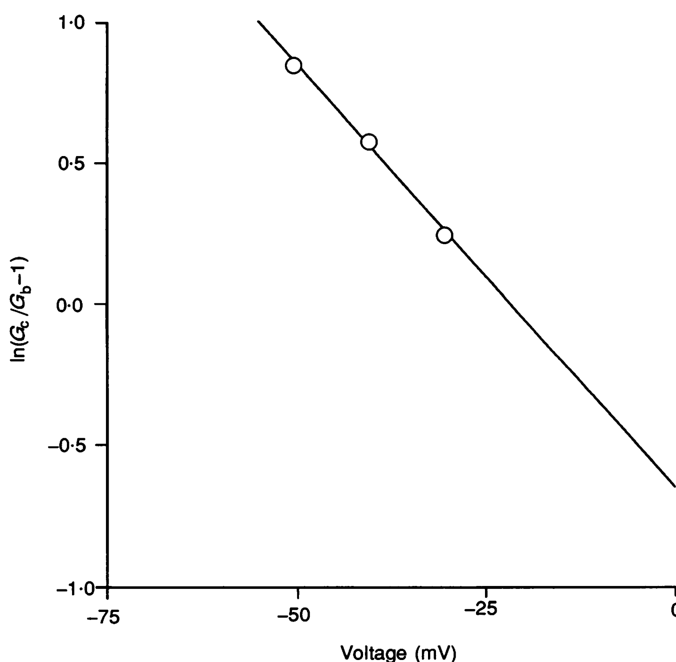

Figure 8. Voltage dependency of BisG10 block
Average data from three separate patches are shown. Data are plotted as relative conductance as a function of voltage in the presence of 0·025 mM BisG10. Data analysis is described in the text.

near the channel mouth. This effect may also explain the different ion concentrations needed to produce conductance saturation in reconstituted, solubilized channels in asolectin liposomes as compared with vesicles fused in neutral phospholipid bilayers (Tomlins & Williams, 1986). Another possible explanation for our failure to see a subconductance state is that the state described in bilayers actually corresponds to a different kinetic rather than conductive state (Gray *et al.* 1985; Tomlins & Williams, 1986). The mean dwell time of the short-lived open state seen in patches of extruded SR is substantially less than a millisecond. We have used a high cut-off frequency (2–5 kHz) in our experiments. The very rapid state transitions we have recorded would not be fully resolved if the data had been filtered at lower frequencies resulting in the appearance of an apparent conductance level in the records that is less that the actual level. It should be noted that Fox (1985) has considered and rejected both a sampling error and an effect of lipid composition to explain the appearance of a subconductance state in his data. Based on his observation that the same two states were seen in membranes with different lipid compositions, under different ionic conditions and at different filter frequencies (10–500 Hz), he concluded that the substate is a robust characteristic of the SR K^+ channel. Whatever the explanation, the channel we have studied does have many important characteristics in common with those described for the SR K^+ channel studied in bilayers. Given these similarities it seems unreasonable to propose that we have identified a new type of SR K^+ channel with only a single conductance state.

Limitations of the preparation

While the preparation we have used would seem to provide a more physiological environment for the channel, it is not without its interpretive difficulties. The process leading to the extrusion of blebs of membrane from contracted skinned fibres is not understood well. Careful ultrastructural and immunochemical studies have shown that the membrane blebs contain SR Ca^{2+}-ATPase and ryanodine receptor proteins (Lewis, Dulhunty, Junankar & Stanhope, 1992). Contributions of transverse tubule or mitochondrial membranes could not be ruled out, however. Evidence supporting the idea that the extruded membrane is predominately of SR origin also comes from an analysis of the types of channels that have been found using this preparation. Palade and co-workers have identified both Ca^{2+} and Cl^- channels in extruded membrane and found that they have characteristics appropriate for SR ion channels but inappropriate for Ca^{2+} or Cl^- channels originating from either surface or mitochondrial membranes (Stein & Palade, 1988; Hals *et al.* 1989). We have also studied the Ca^{2+} channel from this membrane and have confirmed its sensitivity to caffeine, a pharmacological agent known to interact specifically with the SR calcium channel (Wang & Best, 1992). Much of the behaviour of the K^+ channel that we have described in this paper is also consistent with it being of SR origin. The selectivity and pharmacological sensitivity of the channel is, in general, similar to that described for SR K^+ channels incorporated into artificial bilayers but unlike those of surface, transverse tubular, or mitochondrial membrane K^+ channels. Thus, we would argue, as others have before, that the extruded blebs are predominately SR membrane and that contamination by transverse tubule or mitochondrial membrane is minor, if it exists at all. However, we have made no direct measurements to rule out possible contamination and cannot, therefore, entirely discount the possibility that the SR K^+ channel we have studied is affected in some way by a small amount of 'foreign' lipid or protein.

Block by BisG10

The channel was blocked by BisG10, a guanadinium derivative reported to have higher affinity for the SR K^+ channel than the quaternary amine blockers (Miller, 1982; Garcia & Miller, 1984*b*). Our calculated affinity for the blocker at zero voltage was 0·048 mM which is in good agreement with the 0·050 mM reported by Garcia & Miller for block of the bilayer conductance. The voltage dependence of block is given by the effective valency $zd = -0{\cdot}76$. Assuming a valence of 1, this indicates that when added to the bath, BisG10 must traverse 76 % of the electric field before reaching its binding site. The results from bilayers show that the quaternary amines bind to a site about 65 % of the way down the field when added to the *trans* side of the bilayer (the side opposite to the one to which the vesicles are added before fusion). In bilayer experiments, vesicles are thought to fuse such that the *trans* side corresponds to the interior of the SR. Our assumption that patches of extruded SR membrane form with the luminal surface of the SR facing the bath are thus supported by the electrical distance of the BisG10 binding site from the membrane surface.

The voltage dependence of BisG10 block is only about half as steep as that reported by Miller (1982) for quaternary blockers of carbon chain length equal to or greater than nine. Miller has proposed that these long chain blockers bind doubled over so that both ends of the molecule interact with the binding site. Apparently, this is not the case with BisG10 although it does cause the 'flickering' block characteristic of the long chain quaternary amines.

Physiological consequences

Assuming our results are typical of the channel in its native state, we conclude, as others have, that the SR K^+ channel imparts a large, relatively invariant monovalent ion permeability to the SR membrane. This conclusion is based on the fact that, despite rapid gating kinetics, channel open probability remains high except at extremes of potential that are unlikely to develop across the SR membrane. In addition, the channel is not affected by physiological changes in cytoplasmic calcium concentration.

Given the high P_0 and relative insensitivity to both voltage and calcium, our data are consistent with the view that the SR K^+ channel acts as a simple current shunt that prevents significant charge separation during calcium release. The physiological importance of counter-ion flow through the K^+ channel is supported by the observation that calcium release from skinned fibres is diminished in the presence of BisG10 or when less permeable ions are substituted for K^+ (Abramcheck & Best, 1989). Given the importance of K^+ conductance in supporting calcium release from the SR, an accurate estimate of SR membrane permeability is of some interest. A number of investigators have previously made such estimates. Such a calculation requires a value for channel density which, because of uncertainties about the area of membrane in a patch, could not be made from our data. Meissner (1983) has estimated SR K^+ channel density from studies of SR vesicles and reports a value of 50 μm^{-2}. Using this number and the conductance (60 pS) and zero voltage open probability ($P_0 = 0{\cdot}9$) from our results allows us to calculate an SR K^+ conductance of 270 mS cm^{-2} at physiological ion gradients. Labarca & Miller (1981) have published estimates of frog SR K^+ conductance somewhat lower than this (60 mS cm^{-2}) based on their finding of a zero voltage $P_0 = 0{\cdot}25$. The existence of SR K^+ conductances of high magnitude are supported by the recent observation that valinomycin does not lower H^+ movement across the SR during Ca^{2+} release suggesting that the normal SR K^+ conductance in intact muscle is relatively large and sufficient to provide charge neutralization (Pape, Konishi & Baylor, 1992).

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