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Asymmetric Block of a Monovalent Cation-Selective Channel of Rabbit Cardiac Sarcoplasmic Reticulum by Succinyl Choline

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Summary. We have investigated the effect of the skeletal muscle relaxant succinyl choline (SC) on the conduction of potassium ions through a monovalent cation-selective channel present in the cardiac muscle sarcoplasmic reticulum membrane (CSR). This channel has been studied under voltage-clamp conditions following the fusion of purified CSR membrane vesicles with preformed planar phospholipid bilayers. The channel assumes a fixed orientation in the bilayer and displays two conducting states (B. Tomlins, A.J. Williams & R.A.P. Montgomery, 1984, *J. Membrane Biol.* **80**:191–199). SC blocks potassium conductance through the channel in a voltage-dependent manner. Block occurs from both sides of the channel, in both conducting states and is resolved as discrete "flickering" events. Although SC is capable of blocking potassium conductance from both sides of the membrane, block is asymmetric. The zero-voltage dissociation constant for block from the cis side of the membrane is approximately threefold lower than that from the *trans* side. Block from the *cis* side displays a linear dependence on SC concentration for both open states and is competitive with potassium ions at saturating potassium activities, consistent with a single-site blocking model. The degree of SC-induced block is also influenced by membrane surface charge. SC block differs from that previously described for bis quaternary ammonium (bis Qn) compounds such as decamethonium in that SC blocks preferentially from the *cis* side of the channel.

Key Words cardiac sarcoplasmic reticulum · monovalent cation channel · succinyl choline

Introduction

The sarcoplasmic reticulum membranes of mammalian skeletal muscle (Miller, 1978; Coronado et al., 1980; Labarca et al., 1980; Miller, 1982*a*), amphibian skeletal muscle (Labarca & Miller, 1981) and mammalian cardiac muscle (Tomlins et al., 1984) contain voltage-gated monovalent cation-selective channels. Although the exact physiological role of these permeability pathways remains to be established it seems probable that they serve as charge-compensating mechanisms during the calcium ion fluxes associated with excitation-contraction coupling (McKinley & Meissner, 1977; Meissner & McKinley, 1982; Garcia & Miller, 1984).

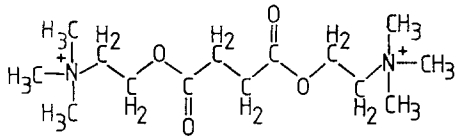
Macroscopic and single-channel properties have been studied under voltage-clamp conditions following the fusion of purified SR membrane vesicles with planar phospholipid bilayers (Miller, 1978; Tomlins et al., 1984). Such experiments have revealed that channels from mammalian skeletal and cardiac muscle SR share many fundamental characteristics including their selectivity and permeability sequences for monovalent cations. In addition, the potassium conductance of both species of channel is blocked by bis Qn compounds such as decamethonium (Coronado & Miller, 1980; Miller, 1982*a*; Tomlins et al., 1984). These and other organic cation blockers have been used as structural probes of the skeletal muscle SR channel. Extensive studies by Miller (Coronado & Miller, 1982; Miller, 1982*a*) have revealed the trans opening of the skeletal muscle SR channel to consist of a large mouth region which narrows to a short constriction approximately 1 nm in length at which ion selectivity occurs. A common blocker binding site exists approximately 65% of the voltage drop across the channel from the *trans* face.

Although a fairly detailed picture of the *trans* opening of the skeletal muscle SR channel has been constructed, little or no information is available on the structure and dimensions of the mouth region or voltage drop from the *cis* face of the channel. The demonstration that potassium conductance through the CSR channel may be blocked when bis Qn compounds are added to the *cis* side of the channel (Tomlins et al., 1984) should enable us to carry out a systematic analysis of this region of the channel. These studies are currently under way.

Here, we report the results of studies of potassium conductance block by SC (I), a bis Qn compound of similar dimensions to decamethonium (II) but differing in the composition of the "hydrocar-

bon" chain linking the two ammonium groups. The presence of oxygen and carbonyl groups in the chain make this region of SC less hydrophobic than the methylene chain of decamethonium.

SC blocks the potassium conductance of the CSR monovalent cation channel from both the *cis* and *trans* sides of the channel. In contrast to decamethonium and related compounds, SC blocks predominantly from the *cis* side of the channel causing well-resolved "flickering" events.



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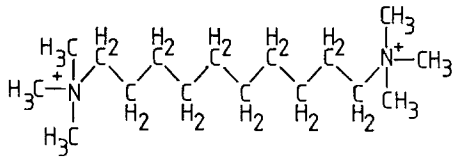

(II)

Materials and Methods

Isolation of Rabbit Cardiac and Skeletal SR Membranes

The isolation of cardiac muscle SR was based on the procedure of Jones and Cala (1981), as described previously (Tomlins et al., 1984). In the experiments described here, only Fraction III (oxalate-loaded material) was used.

Skeletal muscle SR was isolated as described by Garcia and Miller (1984).

Incorporation of Membrane Vesicles into Planar Phospholipid Bilayers and the Measurement of Single-Channel Activity

Both skeletal and cardiac SR membrane vesicles were incorporated into planar phospholipid bilayers by fusion (Miller, 1978). Bilayers were formed from decane solutions of purified phospholipids. Neutral bilayers contained 100% phosphatidyl ethanolamine (PE) (30 mM) and charged bilayers contained either 70% PE, 30% phosphatidyl serine (PS) or 30% PE, 70% PS (30 mM). The bilayer separated two aqueous chambers, one of which, designated *trans*, was held at virtual ground while the other, designated *cis*, could be clamped at a range of holding potentials relative to ground. Current flow through the bilayer was monitored using an operational amplifier as a current-voltage converter (Miller, 1982*b*). The output of the amplifier was displayed on an oscilloscope, stored on FM tape, digitized and analyzed using a Z-80 based microcomputer.

SR vesicles were induced to fuse with negatively charged bilayers as previously described (Tomlins et al., 1984). Membrane vesicles were incorporated into neutral bilayers in the presence of an osmotic gradient but with no added calcium in the *cis* chamber. The final concentration of membrane protein in the cis chamber was 1 to 10 μg/ml. Following channel incorporation unfused vesicles were perfused out. All experiments were carried out at room temperature (20 to 22°C). Unless otherwise indicated experiments were carried out with symmetrical 150 mM K^+ solutions (as sulphate salt), 5 mM HEPES,[1] pH 7.2, in the *cis* and *trans* chambers. Potassium activities were calculated according to Tamamushi and Goto (1970). When SC was present it was added symmetrically to the *cis* and *trans* chambers.

Phospholipids were purchased from Avanti Polar Lipids, Alabama. Succinyl choline chloride was purchased from Sigma Chemical Company.

Analysis of Channel Conductance in the Presence of SC

SC block of K^+ conductance is resolved as "flickering." While the absolute single-channel conductance remains unaltered, the time-averaged conductance (γ) is reduced due to channel flickering. In order to evaluate the time-averaged conductance, data stored on FM tape was low-pass filtered at 100 Hz and sampled every 8 msec using an 8-bit analogue-to-digital converter. A channel opening could then be displayed on the monitor. A level is set and any samples having a value above this level are taken as open, and any with values below are taken as closed. This results in a clear trace allowing only two states, open (unblocked) and blocked. As the samples represent equal time intervals the time-averaged conductance (γ) can be determined by counting the number of open samples (nO) and the total number of samples in the channel opening (nT). The time-averaged conductance is then given by $(nO/nT) \times \gamma_o$, where γ_o = the control (unblocked) conductance.

Results

CSR Channel Activity in Planar Phospholipid Bilayers

Figure 1*a* illustrates the discrete conductance fluctuations normally observed in K^+-containing media following the fusion of CSR membrane vesicles with a neutral planar phospholipid bilayer. Fusion events generally incorporate between 2 and 4 channels. However, the data described for SC block were obtained in experiments in which only a single channel was incorporated into the bilayer. Channel activity can be described in terms of a three-state scheme, in which there is a well-defined closed state (zero conductance) and two open states designated α and β (Tomlins et al., 1984). Typically, the α-state conductance is 60 to 70% of the β-state conductance and is always resolved as a much "noisier" state. In addition, the α state displays a considera-

[1] Abbreviations: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; decamethonium, decamethylenebis (trimethyl) ammonium bromide; Succinyl choline, 2,2'-[(1,4-dioxo-1,4-butanediyl) bis (oxy)] bis [N,N,N,-trimethylethanaminium] di-chloride.

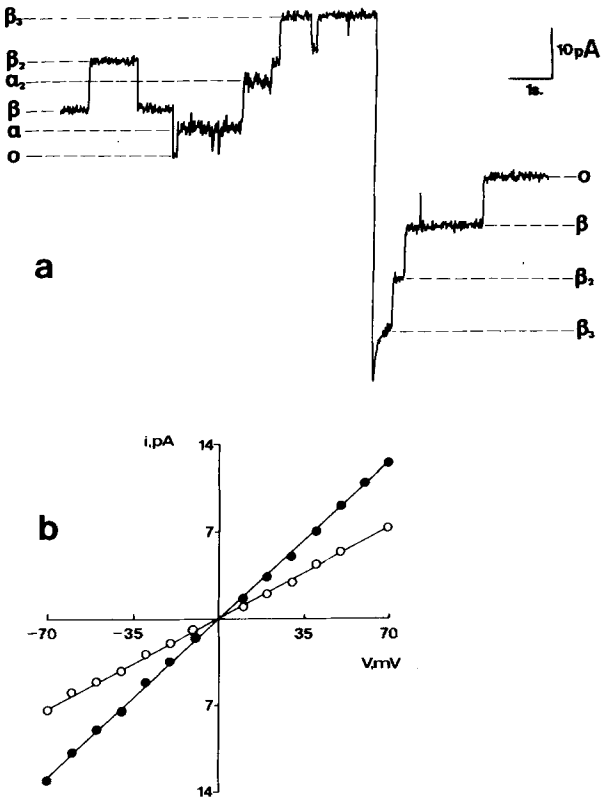

Fig. 1. (*a*) Current fluctuations of cardiac SR channels illustrating the three conductance states, closed (○), α and β; at a holding potential of +50 mV (top trace) and −50 mV (bottom trace). Traces were low-pass filtered at 400 Hz. The switch from +50 to −50 mV produces a large capacitative spike and an increased probability of channel closing. At positive holding potentials increased current flow is indicated by an upward deflection while increased current flow is indicated by a downward deflection at negative holding potentials. (*b*) Single-channel current-voltage relationship for α (○) and β (●) states. Channel fluctuations were recorded after incorporation into a neutral (100% PE) bilayer with symmetrical solutions of 75 mM K_2SO_4, 5 mM HEPES, pH 7.2. Each point represents the mean of five to 10 separate determinations at the indicated holding potential. Lines were drawn by eye. α-state single-channel conductance = 100 pS. β-state single-channel conductance = 184 pS

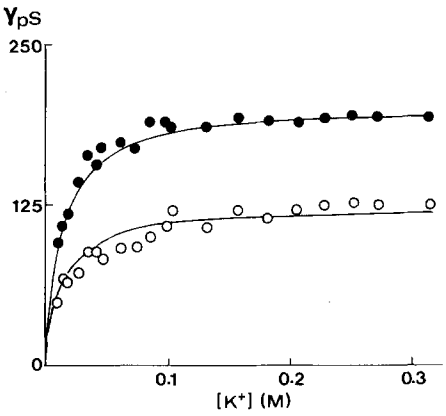

Fig. 2. Conductance-activity relationship in neutral membranes. Channel conductance of α (○) and β (●) states were determined as described in Fig. 1*b*, using K_2SO_4 solutions ranging in concentration from 5 to 300 mM, all containing 5 mM HEPES, pH 7.2. Each point represents a single-channel conductance (γ) obtained from the slope of a current-voltage relationship (as in Fig. 1*b*). All plots were linear over the range ± 70 mV and at all K^+ activities studied. K^+ activity was calculated according to Tamamushi and Goto (1970). The solid curves were drawn according to the equation $\gamma/\gamma_{max} = aK\ (aK + Kd)$, where $\gamma_{max}(\alpha)$ = 124 pS, and $\gamma_{max}(\beta)$ = 204 pS. For both states Kd = 12 mM

bly more variable conductance than the β state. Analysis of single-step transition probabilities has revealed that there is no requirement for the channel to enter the α state during transitions between the closed and β states (Tomlins et al., 1984). This is not the case for the amphibian skeletal muscle SR channel (Labarca & Miller, 1981).

Both mammalian skeletal and cardiac muscle SR monovalent cation-selective channels show voltage-dependent gating. Positive holding potentials favor the opening of the channel, while negative holding potentials increase the probability of channel closing. The single-channel conductance is voltage-independent over the holding potential range −70 to +70 mV (Fig. 1*b*) as has previously been demonstrated in negatively charged bilayers (Tomlins et al., 1984).

Both conductance states of the CSR channel saturate with increasing K^+ activity in neutral phospholipid bilayers (Fig. 2). Conductance follows a simple saturation curve (Kd = 12 ± 2 mM for both conducting states), indicating, as has previously been demonstrated for the skeletal muscle SR channel (Coronado et al., 1980), that the channel can be occupied by no more than one ion at a time.

Block by SC

Following the addition of 1 mM SC to both the *cis* and *trans* chambers, the channel can be seen to undergo rapid transitions between the open states and a blocked state (channel flickering) (Fig. 3). The blocked state conductance level is indistinguishable from the normal closed state but the mean blocked and closed times are obviously quite different.

Channel flickering occurs in both the α and β open states, although flickering appears less common in the α state (Fig. 3). The rate of channel flickering is greater at positive holding potentials than negative holding potentials (Fig. 3). Thus SC is more effective when acting from the *cis* side of the channel. This is in contrast to the action of the bis

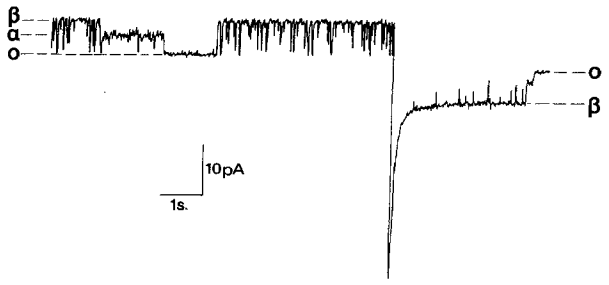

Fig. 3. SC-induced channel flickering of cardiac SR channel. Holding potential is +40 mV (top trace) and −40 mV (bottom trace). Note the reduced frequency of flickering at negative holding potentials. A single cardiac SR channel was incorporated into a neutral bilayer and fluctuations analyzed in 75 mM K_2SO_4, 5 mM HEPES, pH 7.2, containing 1 mM SC. These traces should be compared with data obtained in the absence of SC (Fig. 1*a*)

Qn compounds such as decamethonium which block more effectively from the trans side of both the skeletal and cardiac SR channels (Coronado & Miller, 1980; Miller, 1982*a*; Tomlins et al., 1984).

A quantitative analysis of channel flickering yields data on time-averaged conductance and hence the degree of channel block (*see* Materials and Methods). The degree of K^+ conductance block by SC increases with applied voltage, as is shown in Fig. 4*a*, in which normalized blocked conductance γ/γ_o (where γ = time-averaged conductance in the presence of the blocker and γ_o control conductance) is plotted against applied holding potential. The voltage-dependent nature of the block from both cis and trans sides of the channel implies some interaction between the charged blocker molecule and a site or sites situated within the voltage drop of the channel. For a voltage-dependent block of this type the time-averaged conductance (γ) is given by (Woodhull, 1973)

$$\gamma = \gamma_o[1 + (B/Kb0)\exp(z\delta FV/RT)]^{-1} \quad (1)$$

where B, $Kb0$ and z are the concentration, zero voltage dissociation constant and valence of the blocker, respectively. δ is the portion of the total voltage-drop (V) across the membrane experienced at the site of the blocking reaction. γ_o is the control, unblocked conductance and F, R and T have their usual meanings. The quantity $z\delta$ is usually termed the effective valence of the blocking reaction (Coronado & Miller, 1979). A linearized form of Eq. (1) may be used to obtain the blocking parameters $Kb0$ and $z\delta$ by determining γ as a function of V at constant B (Coronado & Miller, 1979) (Table 1). The data shown in Fig. 4*a* is replotted in the linearized form in Fig. 4*b*. The asymmetric nature of the block

Table 1. Blocking parameters from cardiac and skeletal SR channels obtained in the presence of 1 mM succinyl choline[a]

	Conductance state	$Kb(0)$ mM	$z\delta$	r
(*a*) *Cardiac SR*				
cis block	α	25.70	0.95	0.98
	β	20.70	1.17	0.98
trans block	β	70.60	−0.86	0.96
(*b*) *Skeletal SR*				
cis block	α	34.00	0.89	0.95
	β	19.72	1.05	0.98
trans block	β	58.00	−0.73	0.91

[a] Blocking parameters obtained in the presence of 1 mM SC as described in Fig. 4 for cardiac SR. $z\delta$ values are expressed as a distance into the voltage drop from the side of the membrane to which the drug is added. 'r' values represent the correlation coefficients derived from the least-squares plots of the data.

Data for skeletal SR are taken from four different experiments, and measurements were made over the potential range ±80 mV, exactly as described for cardiac SR (Figs. 1 & 4). Control current/voltage relationships were linear over this range and values for γ_o α state = 95 ± 3.2 pS, and for γ_o β state = 176 ± 5.4 pS (n = 4 ± SD).

The results described above were obtained from lines drawn by least-square regression and contained 15 different potential measurements for *cis* block, while for *trans* block five different potential measurements were used. For each potential between five and 15 separate determinations were made of channel block. The standard deviation for all the data was between 5 and 11%.

by SC, depicted qualitatively in Fig. 3 is confirmed by this data. The β state $Kb0$ for *cis* block being approximately threefold lower than that obtained for *trans* block. Although we have only limited data on *trans* block in the α state, preliminary evidence indicates a value for $Kb0$ well in excess of 100 mM and we, therefore, feel justified in suggesting that SC block in the α-conducting state is also asymmetric. In addition to the asymmetric nature of the SC block, differences exist between the $Kb0$ values of the two conducting states. The affinity for the blocker in the α state being some 30% lower than that for the β state.

To obtain information on the relative position of the blocker binding site within the channel voltage drop it is necessary to have a value for z, i.e. the valence of the blocker or, more precisely, the charge carried by the molecule within the voltage drop. For SC this will obviously depend on the conformation of the molecule within the channel and this question will be considered in detail in the discussion.

It has previously been demonstrated that the cardiac muscle SR monovalent cation channel

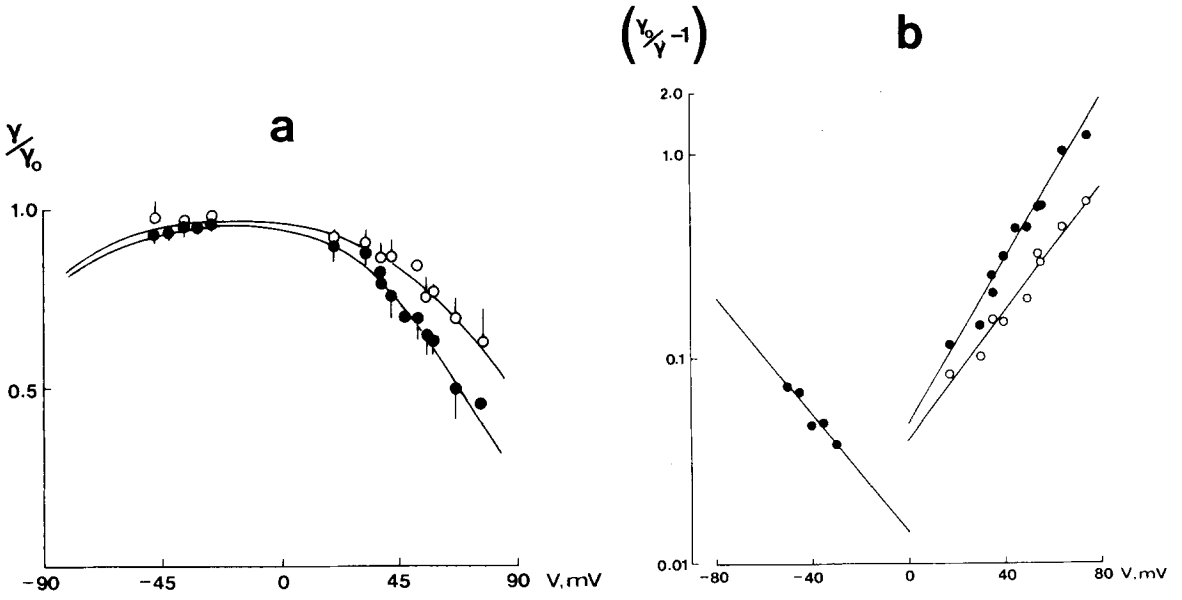

Fig. 4. Voltage dependence of SC block. (*a*) Single-channel K^+ conductance was measured for both α (○) and β (●) states as described in Fig. 1, using neutral phospholipid bilayers in symmetrical solutions of 75 mM K_2SO_4, 5 mM HEPES, pH 7.2, containing 1 mM SC. γ/γ_o = the fraction of the total conductance remaining in the presence of the blocker. γ = reduced K^+ conductance measured in the presence of 1 mM SC, determined as described in Materials and Methods. γ_o = open-state conductance in the absence of blocker (185 pS for the β state, 109 pS for the α state). Each point represents the mean value (± SD) of up to 30 separate determinations at the indicated holding potential. Symbols without error bars have a SD smaller than the diameter of the symbol. Data was obtained from eight different experiments in each of which the bilayer contained only a single channel. The solid curves were drawn assuming a situation in which SC entering the channel from either the *cis* or *trans* chamber binds to an identical site within the voltage drop of the channel, but with different dissociation constants. A formal expression of the voltage-dependent block by SC, for both the α and β states, from both the *cis* and *trans* sides of the channel follows from Eq. (1) and can be written as (Coronado & Miller, 1979; Labarca & Miller, 1981):

$$\gamma/\gamma_o = \left[1 + \frac{[SC]_{cis}}{Kb(0)_{cis}} \cdot \exp\left(z\delta \cdot \frac{FV}{RT}\right) + \frac{[SC]_{trans}}{Kb(0)_{trans}} \exp(z(\delta - 1)) \frac{FV}{RT}\right]^{-1} \quad (2)$$

where z, the valence of the blocker is assumed to be 2 for both the α and β states. The lines have been drawn with the blocking parameters listed in Table 1. Note that δ values are those measured from the *cis* side of the channel. In the α state this is 0.47 ($z\delta$ = 0.945) and for the β state this is 0.59 ($z\delta$ = 1.17). (b) Linearized plot of Eq. (1) for the data in Fig. 4*a*. Error bars have been omitted for clarity and the data for *trans* block of the α state have not been included. Lines were drawn by least-squares regression and the blocking parameters obtained from these plots are listed in Table 1

shares many characteristics with the skeletal muscle SR channel (Tomlins et al., 1984). It was, therefore, of interest to determine if SC blocked K^+ conductance through the skeletal SR channel in a similar manner. By employing identical conditions to those used for cardiac SR, we have obtained very similar blocking parameters for the skeletal SR channel. These parameters are given in Table 1 for comparison.

Influence of Membrane Surface Charge

The data presented in the preceding sections were obtained following the incorporation of channels into neutral (100% PE) bilayers. An interesting observation is that the presence of a negatively charged phospholipid (PS) alters the degree of block by SC in a manner suggesting that the negatively charged head groups in the bilayer are causing an increase of the local concentration of the blocker. The concentration of SC "sensed" by the channel is then higher than that in the bulk solution. The *cis* blocking parameters determined for negatively charged membranes, with symmetrical 1 mM SC are shown in Table 2. A similar effect of surface charge has been reported for *trans* block of the skeletal muscle SR channel by another quaternary ammonium compound "bis Q 11" (Bell & Miller, 1984).

It is apparent from Tables 1 and 2 that the zero-voltage dissociation constant for SC is lower in negatively charged membranes than neutral mem-

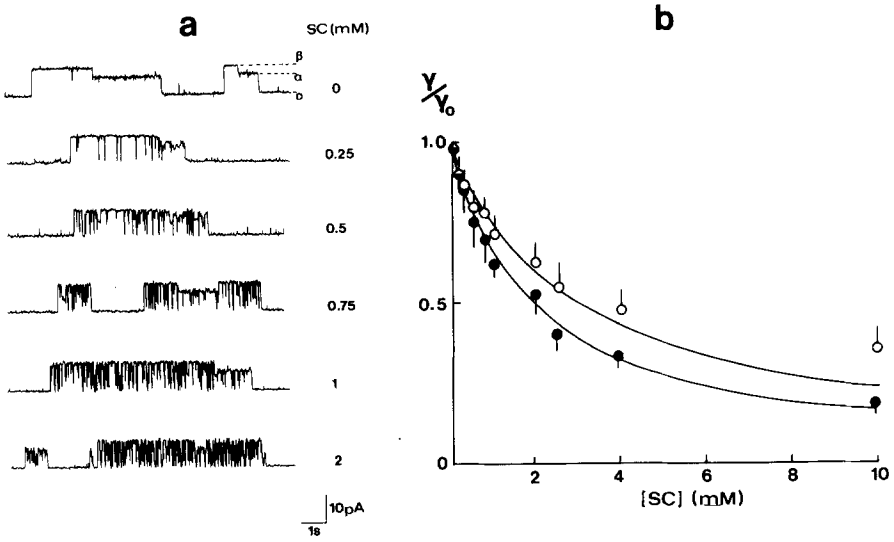

Fig. 5. SC titration of single-channel conductance. (*a*) Representative channel openings at a holding potential of +50 mV in the absence or in the presence of a series of concentrations of SC. Channel fluctuations were monitored following the incorporation of channels into planar bilayers containing 70% PE, 30% PS. *Cis* and *trans* chambers contained 75 mM K_2SO_4, 5 mM HEPES, pH 7.2, and the appropriate concentration of SC. (*b*) Single-channel conductance measurements were made under the conditions described in Fig. 5*a* for both α (○) and β (●) states. Holding potential was +50 mV. SC was present in both *cis* and *trans* chambers, ranging in concentration from 0.01 to 10.0 mM. γ/γ_o is the fraction of the total conductance remaining in the presence of the blocker as described in Fig. 4. Each point represents the mean (± SD) of between 15 and 45 determinations taken from three separate experiments. Symbols without error bars have SD less than the diameter of the symbol. The solid curves were drawn according to:

$$\gamma/\gamma_o = \left[1 + \frac{[SC]}{Kb(V)}\right]^{-1} \tag{3}$$

where γ is the channel conductance at a given SC concentration, γ_o is the channel conductance in the absence of SC and $Kb(V)$ is the dissociation constant for SC at the applied holding potential (+50 mV). The curves were fitted with $Kb(V)\alpha$ = 3.12 mM and $Kb(V)\beta$ = 1.96 mM. These constants were obtained from the linear plot of the data in Fig. 5*b*

branes. However, raising the PS content of the bilayer from 30 to 70% does not appear to reduce the value further. The downward trend of effective valence ($z\delta$) with increasing proportions of PS in the bilayer may be explained by the observations that near a charged surface, a molecule such as SC will not behave as a point-divalent charged particle. On the contrary, its valence (i.e. z) will be between 1 and 2 and will decrease with increasing surface charge (Alvarez et al., 1983), and therefore '$z\delta$' will decrease with increasing surface charge.

Concentration Dependence of SC Block

Because SC blocks predominantly at positive holding potentials (i.e. from the *cis* side of the bilayer) we have confined our investigation to studying blockade from this side. However, SC was added symmetrically to the *cis* and *trans* chambers in all experiments, thereby eliminating possible surface potential effects due to the binding of the blocker to the membrane (Donovan & Latorre, 1979).

A one-site, one-blocker model predicts that at a constant K^+ concentration, increasing the concentration of the blocker should reduce the time-averaged conductance, which should, in turn, follow a single-site inhibition curve. That this prediction holds true for both α and β states is shown in Fig. 5. The apparent dissociation constants, measured at +50 mV, are 1.96 mM for the β state and 3.12 mM for the α state. This result confirms and extends the observation (Figs. 3 & 4) that the α state is less susceptible to SC block than the β state.

Competition of SC with K^+

Throughout this study we have assumed that SC blocks K^+ conduction through the channel by bind-

ing to a site normally involved in K^+ translocation. If this is true, SC binding and hence conductance block, should be competitive with K^+. The data presented in Fig. 6 demonstrate that this is so for both the α and β states at a fixed SC concentration and a range of K^+ concentrations from 0.15 to 0.6 M. At these K^+ concentrations conductance is essentially saturated (*see* Fig. 2). The data is presented in the form of double-reciprocal plots for SC concentrations of 1.0 and 2.5 mM.

It is evident that while the maximum channel conductance is independent of SC concentration, the apparent dissociation constant for K^+ varies with SC concentration, as would be predicted for a one-site competitive scheme. The apparent dissociation constant for SC may be obtained from the slopes of these plots (*see* legend of Fig. 6).

The pure competitive nature of the block by SC is further suggested by the fact that the apparent dissociation constants for K^+ ($Kd_{app.}$) are a linear function of SC concentration (Fig. 6*c*). The true dissociation constant for SC can be obtained from this plot (*see* legend of Fig. 6). For the β state this value is 0.38 mM and for the α state it is 0.75 mM.

Table 2. Effect of lipid charge on blocking parameters[a]

	Conductance state	$Kb(0)$ mM	$z\delta$	r
(a) 30% PS, 70% PE				
cis block	α	11.64	0.83	0.83
	β	11.00	1.12	0.96
(b) 70% PS, 30% PE				
cis block	α	11.27	0.67	0.97
	β	9.69	0.94	0.99

[a] Blocking parameters were obtained in the presence of 1 mM SC as described in Fig. 4, except that charged phospholipid bilayers were used (as indicated) instead of neutral bilayers (100% PE). Although only *cis* block was analyzed, SC was also present (1.0 mM) in the *trans* chamber. Results using neutral bilayers are illustrated in Table 1. Data for (*a*) 30% PS/70% PE were obtained from three separate experiments, and measurements were made over the potential range ±80 mV, exactly as described for neutral bilayers (Figs. 1 & 4). Control current/voltage relationships were linear over this range, and values for γ_o α state = 137 ± 5 pS and for $\gamma_o\beta$ state = 196 ± 9 pS (n = 3 ± SD). Results in the presence of SC were taken from lines drawn by least-square regression and used 17 different potential measurements between 0 and +80 mV. For each potential between five and 30 separate determinations of channel block were made.

Data for (*b*) 70% PS, 30% PE, were obtained from two different experiments, over the potential range ±75 mV, exactly as described in Figs. 1 & 4. Control current/voltage relationships were linear over this range and values for γ_o α state = 137 ± 3 pS and for $\gamma_o\beta$ state = 188 ± 1 pS (mean of two expts.). Results were taken from lines drawn by least-square regression and used 11 different potential measurements. For each potential between five and 15 separate determinations of channel block were made. The standard deviation for all measurements made in the presence of SC was between 5 and 13%.

Discussion

K^+ Conduction

The mechanisms of ion conduction and block of the K^+-selective channel of mammalian skeletal muscle SR have been extensively characterized by Miller and his colleagues. These studies have established that the process of K^+ conduction through this channel is extremely simple and can, in fact, be described by a single-ion model (Läuger, 1973). In accordance with this model, the conductance pathway can be occupied by only one ion, either conducting or blocking, at a time. A wide range of large organic cations have been shown to block K^+ conductance through the channel, the blocking reaction being described by a simple competition between the conducting and blocking ions for binding sites normally involved in K^+ conduction (Coronado & Miller, 1980, 1982; Coronado et al., 1980; Miller, 1982*a*).

Using the methods of Miller, we have previously demonstrated the existence of a similar monovalent cation-selective channel in mammalian cardiac muscle SR (Tomlins et al., 1984). We have established that the two channels have the same selectivity sequence for monovalent cations and that K^+ conductance through the cardiac SR channel may be blocked by bis Qn ions such as decamethonium in a qualitatively and quantitatively similar manner to the skeletal SR channel. The data presented here provide further evidence that the mechanism of ion conduction is similar in the two channels. As would be predicted for a single-ion model, channel conductance of the cardiac SR channel varies with K^+ activity according to a simple "Michaelis-Menten" scheme (Fig. 2). Both conducting states saturate with increasing K^+ activity, half saturation occurring at approximaely 12 mM. Therefore, the mechanism for K^+ conduction appears identical for the cardiac and skeletal channels. However, these experiments do highlight one significant difference. Although the maximal conductance of the two channels is very similar, the cardiac channel has a considerably higher affinity for

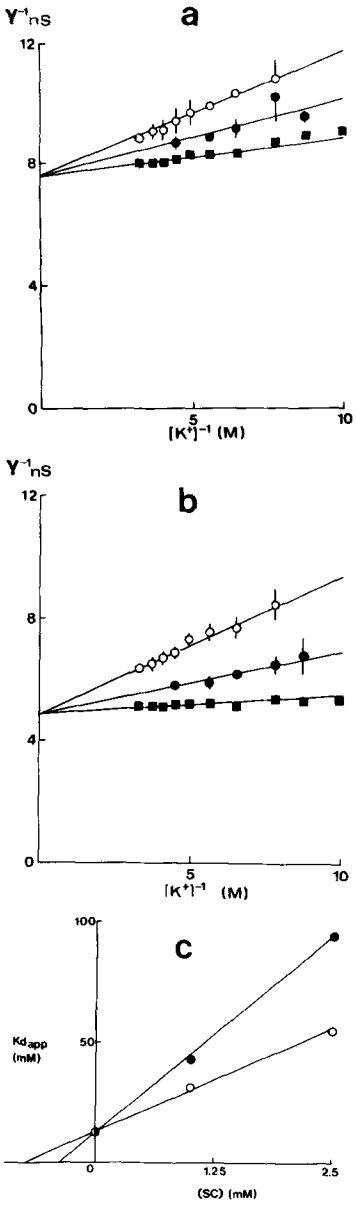

Fig. 6. Competitive block of K^+ conductance by SC. (*a* & *b*) Control K^+ conductance for both the (*a*) α state and (*b*) β state were measured in neutral phospholipid bilayers at different K^+ activities (SO_4^{2-} salt) as described in Fig. 2*a* (■). For each K^+ activity, time-averaged channel conductance (γ) was also determined in the presence of 1.0 mM (●) or 2.5 mM SC (○) present in both *cis* and *trans* chambers, at a holding potential of +50 mV. Each point represents the mean (± SD) of between 5 and 25 determinations, taken from seven separate experiments. Symbols without error bars have SD less than the size of the symbol. Data obtained in this way are plotted in double-reciprocal form and the lines were drawn by eye. The data indicate that the block by SC is competitive with K^+ ions, since the maximal channel conductance is independent of SC, but the dissociation constant for K^+, $Kd(k)$, varies with blocker concentration. From the slope of these lines in the presence of SC it is possible to obtain the apparent dissociation constants for SC (Ki), since for a competitive scheme:

$$\text{Slope} = \left(1 + \frac{a\text{SC}}{Ki}\right)\left(\frac{Kd(\text{K})}{\gamma_{max}}\right) \tag{4}$$

where aSC is the activity of SC, Ki is the apparent dissociation constant for SC, Kd(K) is the apparent dissociation constant for K^+ in the absence of SC and γ_{max} is the maximal channel conductance. In these experiments Kd(K) for both α and β states is 13 mM, while γ_{max} (α) = 132 pS and γ_{max} (β) = 204 pS. Using these parameters we obtain Ki(SC) for the α state of 0.62 and 0.78 mM at 1.0 and 2.5 mM SC, respectively, and for the β state Ki(SC) values of 0.44 and 0.41 mM at 1.0 and 2.5 mM SC, respectively. (*c*) Plot of the apparent dissociation constants for K^+ for both α (○) and β (●) states, against SC concentration. The intercept of the [SC] axis provides the true dissociation constant for SC since:

$$Kd(\text{app}) = \frac{Kd(\text{K}) \cdot [\text{SC}]}{Ki} + (Kd(\text{K})). \tag{5}$$

For the α state this value is 0.75 mM and for the β state it is 0.38 mM. Values for Kd(app) were obtained from the intercepts on the 1/activity axis in Fig. 6*a* & *b*

K^+ than the skeletal SR channel (skeletal Kd = 54 mM; Coronado et al., 1980).

SC-Induced Channel Flickering

Block of K^+ conductance through the cardiac SR channel by SC can be described by a one-site one-blocker model in which the conductance pathway can accommodate either one K^+ ion or one SC ion at a time. In such a situation conductance fluctuates between the normal conducting level and a blocked, nonconducting level. The duration of the blocked state, i.e. the dwell time of the blocker in the channel, is sufficiently long for it to be resolved as channel flickering (Fig. 3).

In previous studies of bis Qn block of the skeletal SR channel (Miller, 1982*a*) two distinct forms of conductance block were observed. Bis Qn molecules with two to eight methylene groups caused smooth conductance block, i.e. blocking events of short duration which could not be resolved as individual events, but produced a time-averaged reduction in channel conductance. Molecules with nine or more methylene groups induced well-defined channel flickering. Transition from smooth to flickering block was accompanied by a doubling in the effective valence of the blocking reaction and an apparent doubling of the dependence of binding affinity on methylene chain length (Miller, 1982*a*). The demonstration that SC causes channel flicker-

ing under conditions in which it has a zero-voltage dissociation constant of > 70 mM (e.g. from the *trans* side of the bilayer) argues that the long dwell time of the blocker in the conduction pathway is not dependent on the affinity of the binding site for the blocker, but may be related to the conformation of the molecule within the channel. Miller (1982*a*) suggested that bis Qn molecules of $n = 9$ or more, block in a bent configuration with the two ammonium groups side by side in the channel, whereas shorter chain bis Qn compounds remain in a linear configuration within the channel.

We believe that molecules which assume a bent configuration and these include SC, induce flickering, while linear blockers do not. This mechanism appears to be independent of binding affinity. A speculative suggestion might then be that a conformational change is necessary for the blocker to leave the conduction pathway, for example the unbending of the molecule, and it would be this requirement which produces long dwell times and hence channel flickering.

Asymmetric Block of K^+ Conductance in SR Channels

A large number of organic cations have been shown to block K^+ conductance of the skeletal muscle SR channel. In all cases block is considerably more effective from the *trans* side of the membrane (Coronado & Miller, 1982; Miller, 1982*a*). We have previously demonstrated that decamethonium blocks K^+ conductance through the cardiac SR channel from both sides of the bilayer, but again block is more effective from the *trans* side of the membrane (Tomlins et al., 1984). We have also obtained blocking parameters very similar to those obtained with the cardiac SR channel for *cis* and *trans* block of the skeletal SR channel by decamethonium (*data not shown*). SC blocks K^+ conductance through both the cardiac and skeletal SR channels in both directions. However, an unusual asymmetry is observed, block being more effective from the *cis* side of the membrane (Figs. 3 & 4, Table 1).

Asymmetric blocker binding affinities are most probably related to differences in the chemical nature of the channel openings at the cis and trans faces of the membrane. Miller (1982*a*) suggested the presence of a "hydrophobic patch" in the trans opening of the skeletal muscle channel to explain the finding that the blocking affinity of the bis Qn compounds increases as the alkyl chain length increases. Our results are consistent with this picture. Decamethonium and SC are molecules of very similar size and flexibility, differing only in the composition of the chain linking the ammonium groups. Oxygen and carbonyl residues of SC make this region of the molecule more hydrophilic than the alkyl chain of decamethonium. We would suggest that this more hydrophilic chain decreases the likelihood of SC being stabilized by the hydrophobic patch of the trans opening of the channel, which, in turn, produces a lower binding affinity.

We can extend our discussion to the cis opening of the channel. In both skeletal and cardiac channels, the binding affinity for decamethonium at the cis side of the channel is lower than that at the trans side. This suggests that, either the cis opening has no hydrophobic area involved in blocker stabilization, or, more likely, a less hydrophobic region of interaction. This suggestion is supported by the nature of the asymmetric block by SC, with the more hydrophilic chain of SC being stabilized in the cis opening.

In summary, we propose that both cis and trans openings of the cardiac and skeletal channels have hydrophobic regions involved in blocker binding and for this reason decamethonium is bound more tightly than SC on both sides of the membrane. However, we feel that the region of the cis opening is less hydrophobic than that of the trans opening.

In addition to the binding affinity, the voltage dependence of SC block is also asymmetric (Fig. 4 & Table 1). δ, the electrical distance at which the blocker binds within the voltage drop, may be determined knowing z, the valence of the blocker. For a molecule such as SC this will obviously depend upon the conformation of the molecule. In the case of block of the β state from the cis side of the membrane, the effective valence data (Table 1) indicate that both charged groups are involved. Thus, we can envisage the molecule in a horseshoe configuration so that both charged groups bind at the same electrical distance into the voltage drop, as has been postulated for long-chain bis Qn compounds (Coronado & Miller, 1980; Miller, 1982*a*; Tomlins et al., 1984). Alternatively, the molecule may assume an "S"-shaped configuration, in which the carbonyl groups are situated in close proximity to the positively charged tertiary ammonium groups. Again, both charged groups would bind at approximately the same electrical distance into the voltage drop. In the case of cis block, δ would be approximately 0.59, implying a blocker binding site approximately 59% of the distance across the voltage drop from the cis side of the bilayer.

If we assume that SC blocks in a similar bent configuration from the trans side of the channel, i.e. with $z = 2$, it appears that the molecule is binding to an equivalent site to that accessible from the *cis* side of the channel, that is 43% down the voltage

drop from the *trans* side of the membrane (Table 1). The site of SC binding appears to be different to that previously described for bis Qn blockers in the skeletal and cardiac muscle channels (Coronado & Miller, 1980; Miller, 1982*a*; Tomlins et al., 1984).

Based on conduction saturation data (Fig. 2), we have concluded that K^+ conductance through the cardiac SR channel can be described in terms of a single ion scheme. This view is supported by the relationship between time-averaged conductance and blocker concentration, which follows a single-site inhibition curve (Fig. 5) and the simple competitive interaction of SC and K^+ at saturating K^+ activities (Fig. 6).

Nature of the Sub-conductance State, α

The discussion up to now has dealt largely with the fully open, β, conducting state. We will now discuss the nature of the α-state conductance, drawing together evidence from various experiments. Sub-conductance states have been documented for a wide range of channels, including the K^+-selective channel of amphibian skeletal muscle SR (Labarca & Miller, 1981). Other multi-state channels include the acetylcholine receptor (Hamill & Sakmann, 1981; Auerbach & Sachs, 1983), the Ca^{2+}-activated K^+ channel of skeletal muscle sarcolemma (Moczydlowski & Latorre, 1983), of macrophages (Gallin, 1984) and of Hela cells (Sauvé et al., 1983), the inward rectifier K^+ channel of cardiac ventricular cells (Sakmann & Trube, 1984), the chloride channel of *torpedo* electroplax (Miller, 1982*b*; Hanke & Miller, 1983; Miller & White, 1984), neuronal GABA and glycine-activated chloride channels (Hamill et al., 1983) and the chloride channels recently described in Schwann cells (Gray et al., 1984).

In two cases, the *torpedo* chloride channel and the cardiac inward rectifier, it has been suggested that the sub-conductance states represent monomer conductance pathways of polymeric channels. Convincing evidence supports the theory that the *torpedo* chloride channel is a dimer (Miller, 1982*b*; Miller & White, 1984), whereas the cardiac inward rectifier may consist of four identical conductance sub-units (Sakmann & Trube, 1984). The nature of the sub-conductance states of the other channels listed above has not been documented.

Previously, we have demonstrated that both conductance states of the cardiac SR channel display the same monovalent cation selectivity sequence and are blocked to a common, nonconducting level by decamethonium, implying that they share a common conductance pathway. Additionally, we have established that transitions between the β, α and closed states are a random occurrence (Tomlins et al., 1984). In this report we have studied K^+ conductance and SC block of both the β- and α-conducting states. Although the maximal conductances are different, the two states have approximately the same affinity for K^+ (Fig. 2) as one might expect for a common conductance pathway.

The observation that the α state was always resolved as a much noisier state (Fig. 1), suggests the possibility that this state might represent a time-averaged conductance, generated by rapid conductance fluctuations between the fully open (β) state and the closed state. Thus, during the α state the channel would spend 30 to 40% of the observed open time in the closed state. The difference between the conducting states would then be one of gating rather than conduction. What evidence do we have to support this claim? Consider conductance block by SC. If as we suggest the α state is a time-averaged conductance during which the channel is closed for 30 to 40% of the apparent open period, we would predict that, as the channel has to be open for SC to block conductance, the degree of block, observed in the α state, would be equivalently less than that seen in the β state. An inspection of a number of openings in the presence of SC gives qualitative support to this contention (Figs. 3 & 5). Blocking events appear to be less common in the α state than the β state. A quantitative analysis of this phenomenon adds further support. The number of blocking events per unit time was determined for α and β states at +50 mV in the presence of 150 mM K^+ and 1 mM SC, over a total open time of 100 sec. Under these conditions, we observed 15.9 blocking events/sec in the α state and 24.3 blocking events/sec in the β state. Therefore, the blocking rate in the α state is 64% of that seen in the β state. The decreased probability of block in the α state relative to the β state is reflected in the lower $Kb(o)$ for SC in the α state than the β state (Table 1) and the higher apparent and true dissociation constants for SC of the α state (Figs. 5 & 6). Our "gating" hypothesis does in fact predict that the ratio of the dissociation constants of SC in the α and β states should be the same as the ratio of the unblocked conductances, i.e.

$$Kd^{\alpha}/Kd^{\beta} = \gamma_o^{\beta}/\gamma_o^{\alpha}.$$

The results in Table 1, and those in Figs. 5 and 6 confirm this prediction.

We feel that these findings support the suggestion that the sub-conductance state of the cardiac SR channel is a different gating state rather than a different conducting pathway. This hypothesis will be further tested using a range of blocking agents.

The effective valence parameters shown in Tables 1 and 2 imply that the position of the SC binding site within the voltage drop is different in the α- and β-conducting states. Assuming a valence of 2, SC would bind at a site approximately 47% across the voltage drop from the cis side of the channel in the α state, while the binding site in the β state is approximately 59%. This difference could be due to a conformational change in the channel protein, leading to a displacement of the binding site within the voltage drop, rather than signifying two different SC binding sites in the two states. Alternatively, the difference may be the result of an alteration in the field configuration around the binding site during α/β transition (Labarca & Miller, 1981).

What determines the conducting state of the channel? Although we have no firm evidence concerning this question, two observations may point to some electrostatic effect being important: (i) the likelihood of the channel being in the α state is higher in highly charged bilayers than in neutral bilayers and (ii) in neutral bilayers, the likelihood of observing the α state decreases with increasing K^+ activity. However, these observations are purely qualitative and await rigorous investigation.

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