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The Characterization of a Monovalent Cation-Selective Channel of Mammalian Cardiac Muscle Sarcoplasmic Reticulum

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Summary. Rabbit cardiac muscle sarcoplasmic reticulum (SR) was isolated and separated into ryanodine-sensitive and -insensitive fractions (L.R. Jones and S.E. Cala, *J. Biol. Chem.* **256**:11809–11818, 1981). Vesicles of cardiac SR were incorporated into planar phospholipid bilayers by fusion and the channel activity of the membrane studied under voltage-clamp conditions (C. Miller, *J. Membrane Biol.* **40**: 1–23, 1978). Both fractions contain a monovalent cation-selective three-state channel. In the presence of 75 mM K_2SO_4, the fully open state (β) conductance of this channel is 157.2 ± 30 pS and the sub-state (α) conductance is 100.7 ± 21 pS. Both open states display the same selectivity sequence for monovalent cations, i.e. $K^+ > NH_4^+ > Rb^+ > Na^+ > Li^+$ and may be blocked by the skeletal muscle relaxants decamethonium and hexamethonium. Block occurs when the compounds are added to either side of the membrane. The properties of the cardiac SR cation channel are compared with those of the previously reported monovalent cation-selective channels of mammalian and amphibian skeletal muscle SR.

Key Words cardiac sarcoplasmic reticulum · K^+ channel · ion selectivity · decamethonium block

Introduction

The existence of a monovalent cation permeability pathway in the SR of mammalian skeletal muscle was first demonstrated using isotope flux measurements. It was suggested that this system could act as a charge-compensating mechanism countering charge movements associated with the Ca^{2+} fluxes involved in excitation-contraction coupling (McKinley & Meissner, 1977). Subsequently it was demonstrated that the monovalent cation permeability pathway of skeletal muscle SR resided in a voltage-gated potassium-selective channel (Miller, 1978). This channel has now been extensively investigated and characterized in mammalian (Coronado & Miller, 1979, 1980, 1982; Coronado, 1980; Labarca, Coronado & Miller, 1980; Coronado, Rosenberg & Miller, 1980; Miller, 1982*a*) and amphibian (Labarca & Miller, 1981) skeletal muscle SR preparations.

More recently, Meissner and McKinley (1982) have used isotope flux, light scattering and membrane potential measurements to demonstrate that vesicles of SR isolated from mammalian cardiac muscle also contain "putative ion-conducting structures" allowing the flux of K^+, Na^+, H^+ and Cl^-, while being impermeable to Ca^{2+}, Mg^{2+}, choline$^+$, large monovalent anions and glucose.

In this paper we provide evidence that the monovalent cation permeability of cardiac SR is dependent upon the functioning of a voltage-gated channel. The channel has been characterized following the incorporation of vesicles of rabbit cardiac SR into planar phospholipid bilayers (Miller, 1978) and we compare its properties to those of the skeletal muscle SR system. Cardiac SR may be separated into two functionally different fractions, the ryanodine-sensitive and ryanodine-insensitive vesicle populations described by Jones and Cala (1981); both fractions contain the monovalent cation-selective channel.

Materials and Methods

Isolation of Rabbit Cardiac SR

The isolation procedure used was based on the procedure of Jones and Cala (1981). Adult male New Zealand White rabbits were killed with a blow to the head. The hearts were rapidly excised and placed in ice-cold 300 mM mannitol, 5 mM HEPES, 0.1 mM EDTA, pH 7.2.[1] The coronary vasculature was flushed

[1] *Abbreviations:* Tris, tris(hydroxymethyl) aminomethane; EDTA, ethylenediaminetetra-acetic acid; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; $NaPP_i$, tetra sodium pyrophosphate; EGTA, ethyleneglycol-bis-(B-aminoethyl-ether)N,N′-tetra acetic acid; decamethonium bromide, decamethylenebis(trimethyl) ammonium bromide; hexamethonium bromide, hexane 1,6-bis(trimethyl) ammonium bromide.

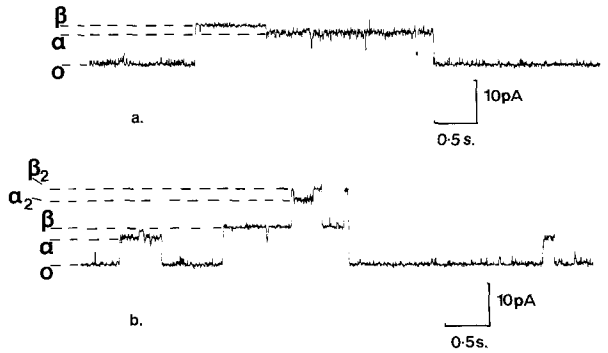

Fig. 1. Single cardiac SR channel current fluctuations showing three conductance states; closed (O), α and β. *a*) holding potential = +60 mV, one channel; *b*) holding potential = +60 mV, two channels. 75 mM K_2SO_4, 10 mM HEPES, 10 mM Tris, 0.1 mM Tris/EDTA, pH 7.2, *cis* and *trans*. An increase in current is indicated by an upwards deflection of the trace

clear of blood by retrograde perfusion through the aorta with the same solution. The auricles and fat were trimmed away and the ventricles opened and flushed clear of any remaining blood. The ventricular tissue was minced with a razor blade; suspended in 10 mM $NaHCO_3$ and homogenized for 3 × 30 sec using a Polytron (PT10/35) homogenizer at setting 5. The homogenate was centrifuged at 14,000 × *g* for 20 min in an MSE High Speed 18 centrifuge to remove cell debris. The supernatant was collected and a vesicular fraction sedimented at 45,000 × *g* for 30 min in an MSE Superspeed 65 centrifuge. The pellet was resuspended in 0.6 M KCl, 30 mM histidine, pH 7.0, and sedimented at 45,000 × *g* for 30 min.

The KCl-washed vesicles were then Ca^{2+} oxalate-loaded, as described by Jones et al. (1979). Following Ca^{2+} oxalate-loading, vesicles were collected by sedimentation at 100,000 × *g* for 30 min and the resulting pellet was resuspended in (mM): 250 sucrose, 300 KCl, 50 $NaPP_i$, 100 Tris, pH 7.2. This material was layered over a discontinuous sucrose gradient composed of 0.6 and 1.5 M sucrose, both sucrose solutions containing 300 mM KCl, 50 mM $NaPP_i$, 100 mM Tris, pH 7.2, and sedimented at 150,000 × *g* for 2 hr. A very light colored band of material (Fraction I) was found at the interface of 0.25 and 0.6 M sucrose. This fraction is predominantly sarcolemmal in origin (Jones et al., 1979). Denser SR vesicles sedimented through the 0.6 M sucrose and formed two distinct populations. One (Fraction II) formed a brown band of material at the interface of 0.6 and 1.5 M sucrose (ryanodine-sensitive SR; Jones & Cala, 1981). The other SR fraction (ryanodine-insensitive; Jones & Cala, 1981) consisted of Ca^{2+} oxalate-loaded material and formed a white "gritty" pellet (Fraction III). Fractions I, II and III were collected, washed in 150 mM KCl, 5 mM HEPES, pH 7.2, and resuspended in 0.4 M sucrose, 5 mM HEPES, pH 7.2, at a concentration of approximately 2 to 4 mg protein/ml. Aliquots were frozen in liquid nitrogen and stored at −80°C.

Incorporation of Cardiac Membrane Vesicles into Planar Phospholipid Bilayers and Measurement of Channel Activity

Cardiac membrane vesicles were incorporated into planar phospholipid bilayers by fusion (Miller, 1978). Bilayers consisted of 30 mM phospholipid (70% phosphatidylethanolamine, 30% phosphatidylserine purchased from Avanti Polar Lipids, Alabama) in decane. 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 voltages 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 this amplifier was filtered at 500 Hz using a six-pole Bessel low-pass filter, displayed on an oscilloscope, stored on FM tape and analyzed using a Z-80 based microcomputer.

The conditions we have used to induce fusion of cardiac membrane vesicles with a preformed bilayer are based on those reported by others to favor the fusion of SR with preformed bilayers (Miller & Racker, 1976). Fusion is favored by the presence of negatively charged phospholipids in the bilayer, divalent cations on the side of the bilayer to which vesicles are added (*cis*) and an osmotic gradient across the bilayer with the *cis* side hyperosmotic to the *trans* side. The specific conditions which we used are as follows: the bilayer contained 30% negatively charged phospholipid (phosphatidylserine); the *cis* chamber contained (mM): 450 KCl, 0.1 EDTA, 1.0 $CaCl_2$, 5 Tris/HCl, pH 7.2; the *trans* chamber contained (mM): 150 KCl, 0.1 EDTA, 5 Tris/HCl, pH 7.2. Cardiac membrane vesicles were added to the *cis* chamber and stirred. Experiments were carried out at 25°C. Following the required channel incorporation, further fusion was prevented by chelating the Ca^{2+} in the *cis* chamber with 1 mM Tris-EGTA. Unfused vesicles were then perfused out.

Results

Incorporation of Rabbit Cardiac SR Vesicles (Fractions II and III) into Planar Phospholipid Bilayers

The incorporation of cardiac SR vesicles into the preformed lipid bilayer was normally accompanied by a small stepwise increase in the background conductance. No large fusion spikes, which have been reported to occur with skeletal SR (Labarca et al., 1980; Labarca & Miller, 1981), were observed. Typically, 1 to 5 channels were incorporated with each fusion event. Both Fractions II and III fused equally well with the bilayer and both populations exclusively yielded the same species of channel. Following fusion we observed discrete conductance fluctuations which corresponded to openings and closings of single channels. Typical fluctuations are shown in Fig. 1. We interpret this behavior as resulting from the opening and closing of a three-state channel with a clearly defined zero conductance or closed state and two open states; a fully open β state with a conductance of about 160 pS and a noisy sub or α state with a conductance of about 100 pS. (The possibility that the observed fluctuations result from the independent opening and closing of two separate channels, one of conductance 100 pS and one of conductance 160 pS or alternatively, one

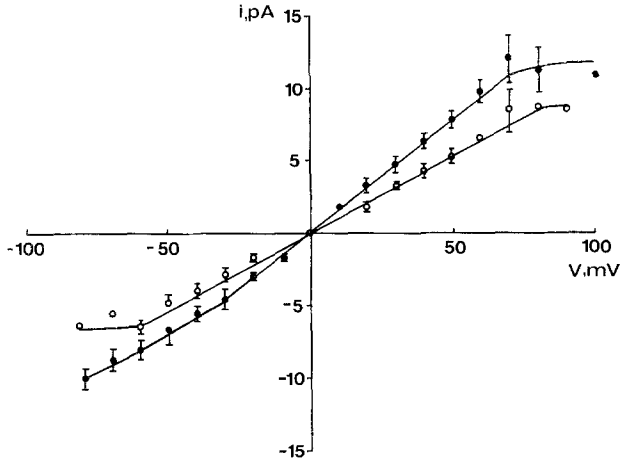

Fig. 2. Single-channel current/voltage relationship for both α(○) and β(●) states in symmetrical solutions of 75 mM K_2SO_4, 10 mM HEPES, 10 mM Tris, 0.1 mM Tris/EDTA, pH 7.2. Points are mean ± standard deviation for eight different membranes and the lines were drawn by least-squares regression

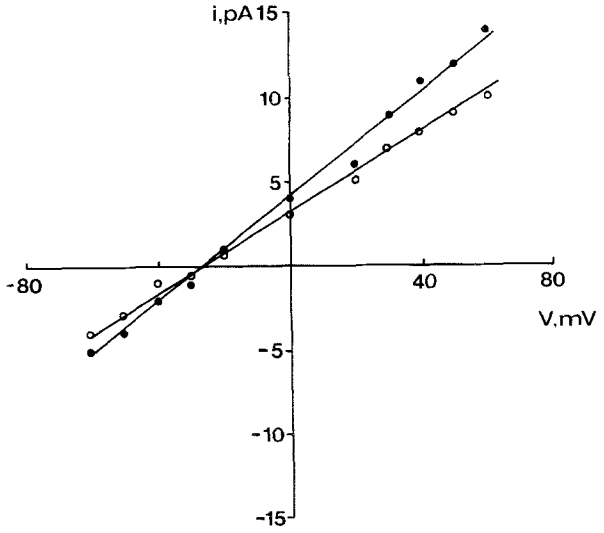

Fig. 3. Single-channel current/voltage relationships for the α(○) and β(●) states of a single cardiac SR channel with a 3:1 *cis/trans* imposed KCl gradient. Current fluctuations were monitored with 150 mM KCl *trans* and 450 mM KCl *cis*, both solutions buffered to pH 7.2 with 10 mM HEPES. The reversal potential for both open states is approximately −26 mV. Lines were drawn by least-squares regression

of 60 pS and one of 100 pS, cannot be totally excluded, but we feel that a considerable body of evidence argues against it. This evidence is detailed in the following sections.) The monovalent cation-selective channel of frog skeletal muscle has been shown to behave as a 3-state channel with an α-state conductance of 50 pS and a β-state conductance of 150 pS. Based on single-step transition probabilities it was concluded that this channel could only fluctuate between the closed and fully open (β) state through the α state (Labarca & Miller, 1981). Using the same method of analysis we have calculated the probabilities of transitions between closed and α, α and closed, α and β, β and α, closed and β and β and closed. Based on 849 individual transitions at holding potentials of +40 and +60 mV, we have obtained data indicating that the probabilities for all the possible transitions are very similar (0.169 ± 0.031 mean ± SD). Therefore, there appears to be no requirement for the cardiac muscle SR channel to enter the α state during transitions between the closed and β states.

Voltage Dependence

The relationship between single-channel current and holding potential is depicted in Fig. 2. For both the α and β states there is an apparent linear relationship between open-state current and holding potential over the range of ±60 mV. The mean conductances of the α and β states in the presence of 75 mM K_2SO_4 are 100.7 ± 21 pS and 157.2 ± 30 pS, respectively (mean ± SD). These values were calculated from transitions in eight different membranes at holding potentials in the range ±60 mV. At holding potentials outside ±60 mV slight rectification becomes apparent (Fig. 2).

Although the conductance of both the α and β open states remains virtually constant with holding potential, the probability of the channel being in the open state does vary. As with the channels of mammalian and amphibian skeletal muscle SR, the open states of the cardiac SR channel are favored at positive holding potentials.

Ionic Selectivity—Monovalent Cation Versus Monovalent Anion

The relative permeabilities of monovalent cations and anions through the cardiac SR channel may be determined by monitoring the single-channel reversal potential in the presence of an ionic gradient across the bilayer (Coronado et al., 1980). The results of such an experiment are shown in Fig. 3. Single-channel current flow was determined over a range of holding potentials with a 3 : 1 KCl gradient imposed across the bilayer (*cis*: 450 mM KCl, 0.1 mM EDTA, 10 mM HEPES, pH 7.2; *trans:* 150 mM KCl, 0.1 mM EDTA, 10 mM HEPES, pH 7.2). The measured reversal potential of both the α and β states of −25.5 ± 1.7 mV (mean ± SD, n = 7) is in good agreement with that calculated from the

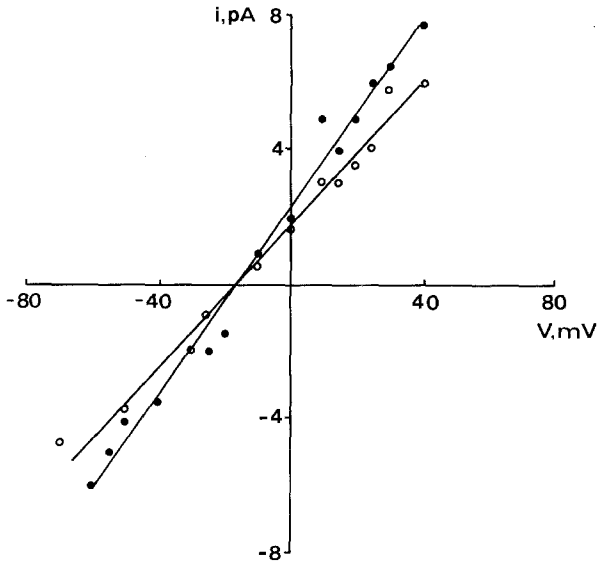

Fig. 4. Single-channel current/voltage plot drawn by least-squares regression from a single bi-ionic reversal potential experiment using 250 mM Na_2SO_4 in the *trans* chamber and 250 mM K_2SO_4 in the *cis* chamber, both solutions buffered with 10 mM HEPES to pH 7.2; α(○), β(●). Bi-ionic experiments were carried out as follows: SR vesicles were fused in symmetrical solutions of *a*) 250 mM $(NH_4)_2SO_4$, 10 mM HEPES, pH 7.2; *b*) 250 mM Na_2SO_4, 10 mM HEPES, pH 7.2; *c*) 250 mM Li_2SO_4, 10 mM HEPES, pH 7.2; or *d*) 500 mM RbCl, 10 mM HEPES, pH 7.2. After fusion, the *cis* chamber solution was perfused out and replaced with either 250 mM K_2SO_4, 10 mM HEPES, pH 7.2 (*a, b* and *c*) or 500 mM KCl, 10 mM HEPES, pH 7.2 (*d*). Permeability ratios were calculated for ion X^+ using $PK^+/PX^+ = aX^+/aK^+ \exp - FE/RT$ where F = the Faraday constant, R = the gas constant, T = the absolute temperature, E = the reversal potential, and a = ion activity (Robinson & Stokes, 1955). The following permeability ratios were obtained from single representative experiments: $PK^+/PNH_4^+ = 1.04$, $PK^+/PNa^+ = 1.94$, $PK^+/PLi^+ = 2.6$, and $PK^+/PRb^+ = 1.14$

Nernst equation for an ideally monovalent cation-selective permeability pathway in the presence of a 3:1 gradient, i.e. −28.5 mV. Therefore, we may conclude that both the α and β states of the cardiac SR channel are predominantly selective for monovalent cations (K^+) rather than monovalent anions (Cl^-).

Monovalent Cation Selectivity Sequence

Monovalent cation permeability ratios were determined from bi-ionic reversal potential measurements (Coronado et al., 1980) with 500 mM K^+ in the *cis* chamber and either 500 mM Na^+, Rb^+, NH_4^+ or Li^+ in the *trans* chamber (Fig. 4). Both the α and β open-state measurements yielded identical reversal potentials for a particular bi-ionic condition.

Table 1.

Ion X^+	Conductance (pS)		gK^+/gX^+ (β)	PK^+/PX^+ (β)
	Full open state (β)	Sub-state (α)		
K^+	218.0 ± 20.6	145.7 ± 30.0	1.00	1.00
NH_4^+	156.4 ± 23.2	97.0 ± 28.8	1.40	1.04
Rb^+	117.0 ± 30.3	88.0 ± 30.2	1.87	1.14
Na^+	72.0 ± 13.2	53.0 ± 10.5	3.04	1.94
Li^+	26.7 ± 2.4	Unmeasurable	8.19	2.60

Selectivity parameters for several monovalent cations measured from single-channel openings of the cardiac SR monovalent cation-selective channel. α and β conductances (mean ± SD, $n \geq 16$) were calculated from single-channel events occurring at holding potentials between ±80 mV with symmetrical solutions of 500 mM X^+. PK^+/PX^+, permeability ratios from single representative experiments were determined under bi-ionic conditions as described in Fig. 4.

Based on the data obtained in these experiments, the following selectivity sequence may be constructed; $K^+ \geq NH_4^+ > Rb^+ > Na^+ > Li^+$.

Additional information was obtained by monitoring the conductance of both the α and β states in the presence of symmetrical 500 mM solutions of the various monovalent cations. Both α and β states displayed the same sequence of conductance, $K^+ > NH_4^+ > Rb^+ > Na^+ > Li^+$. The monovalent cation selectivity parameters that we have determined are summarized in Table 1.

Block by Cholinergic Drugs

K^+ current through the mammalian skeletal muscle SR channel is blocked by decamethonium and hexamethonium (Coronado & Miller, 1980). Hexamethonium block is characterized by an apparent smooth decrease in single-channel conductance, while decamethonium blocks with a far greater affinity and induces channel flickering, which almost certainly represents the rapid transitions between the fully open and blocked states of the channel (Coronado & Miller, 1980). Subsequently, Miller has used a series of bisquaternary ammonium blockers of varying chain length to reveal a considerable amount of information about the structure of the channel and the location of the voltage drop within the channel (Miller, 1982*a*).

Both decamethonium and hexamethonium block K^+ current through the monovalent cation channel of cardiac muscle SR. As with the mammalian skeletal muscle SR channel, hexamethonium produces an apparently smooth reduction in single-

Fig. 5. Oscilloscope traces showing decamethonium-induced channel 'flickering'. *a*) Holding potential switched from +40 to −40 mV producing a large capacitive spike. Note the increased frequency of flickering at negative holding potentials, one channel, 75 mM K_2SO_4, 10 mM HEPES, pH 7.2, containing 200 μM decamethonium bromide *cis* and *trans*. An increased current flow is indicated by an upward deflection at +40 mV and a downwards deflection at −40 mV. *b*) Holding potential = +60 mV, decamethonium induces qualitatively similar flickering in both α and β states, two channels, 75 mM K_2SO_4, 10 mM HEPES, pH 7.2, containing 100 μM decamethonium bromide *cis* and *trans*. These traces should be compared with the prolonged openings seen in the absence of the blocker (Fig. 1)

channel conductance (*data not shown*), whereas decamethonium induces channel flickering (Fig. 5). Both compounds will block K^+ current through the cardiac muscle SR channel from either side of the membrane at suitable holding potentials. That is, the positively charged blocking molecules will block from the *trans* side of the membrane at negative holding potentials when the blocker is driven into the channel and similarly, they will block from the *cis* side of the membrane at positive holding potentials. The degree of block increases with increasing concentrations of both compounds (*data not shown*).

Using decamethonium, we have determined a number of blocking parameters. Experiments were carried out using membranes containing only one or two channels incorporated as described in Materials and Methods. Both *cis* and *trans* chambers contained 75 mM K_2SO_4, 0.1 mM EDTA, 10 mM HEPES, pH 7.2, plus 200 μM decamethonium. Single-channel fluctuations were recorded at a range of holding potentials and time-averaged conductance determined using the microcomputer (*see* legend of Fig. 6). To simplify the analysis, openings containing α open states were not analyzed, although flickering in both α and β states appeared qualitatively identical (Fig. 5).

Table 2.

	$K(0)$ (mM)	$z\delta$
Cis block	2.11	0.54
Trans block	1.48	−1.08

Blocking parameters obtained in the presence of 200 μM decamethonium as described in Fig. 6. $z\delta$ values are expressed as a distance into the voltage drop from the side of the membrane to which the drug is added. The parameters for the *trans* block are in good agreement with those previously reported from the *trans* block of the mammalian skeletal muscle SR channel ($K(0)$ = 1.2 ± 0.2 mM; $z\delta$ = −1.22 ± 0.08; Coronado & Miller, 1980).

Decamethonium block of the cardiac SR channel is voltage dependent. However, the voltage dependence of the block appears to be different from the *trans* and *cis* sides of the membrane (Fig. 6). The voltage dependence of the block may be quantified by determining the effective valence $z\delta$ of the blocking reaction (Coronado & Miller, 1979, 1980; Miller 1982*a*).

The time-averaged β open-state conductance (γ) is given by:

$$(\gamma/\gamma 0) = [1 + (B/K(0)) \exp(z\delta FV/RT)]^{-1} \quad (1)$$

where, $K(0)$, B and z are the zero voltage dissociation constant, concentration and valence of the blocker, respectively. δ is the portion of the total voltage drop across the membrane V, experienced at the site of the blocking reaction. $\gamma 0$ is the conductance in the absence of blocker and F, R and T have their usual meanings. A linearized form of Eq. (1) (Fig. 6*b*) may then be used to obtain K(0) and $z\delta$ by determining γ as a function of V at a constant $[B]$ (Coronado & Miller, 1980).

The values obtained from such an analysis with 200 μM decamethonium in both the *cis* and *trans* chambers are presented in Table 2. $K(0)$ and $z\delta$ for block from the *trans* side of the bilayer are in reasonably good agreement with the data previously obtained for the mammalian skeletal muscle SR channel. No data is available for block from the *cis* side of the membrane with the skeletal muscle SR channel.

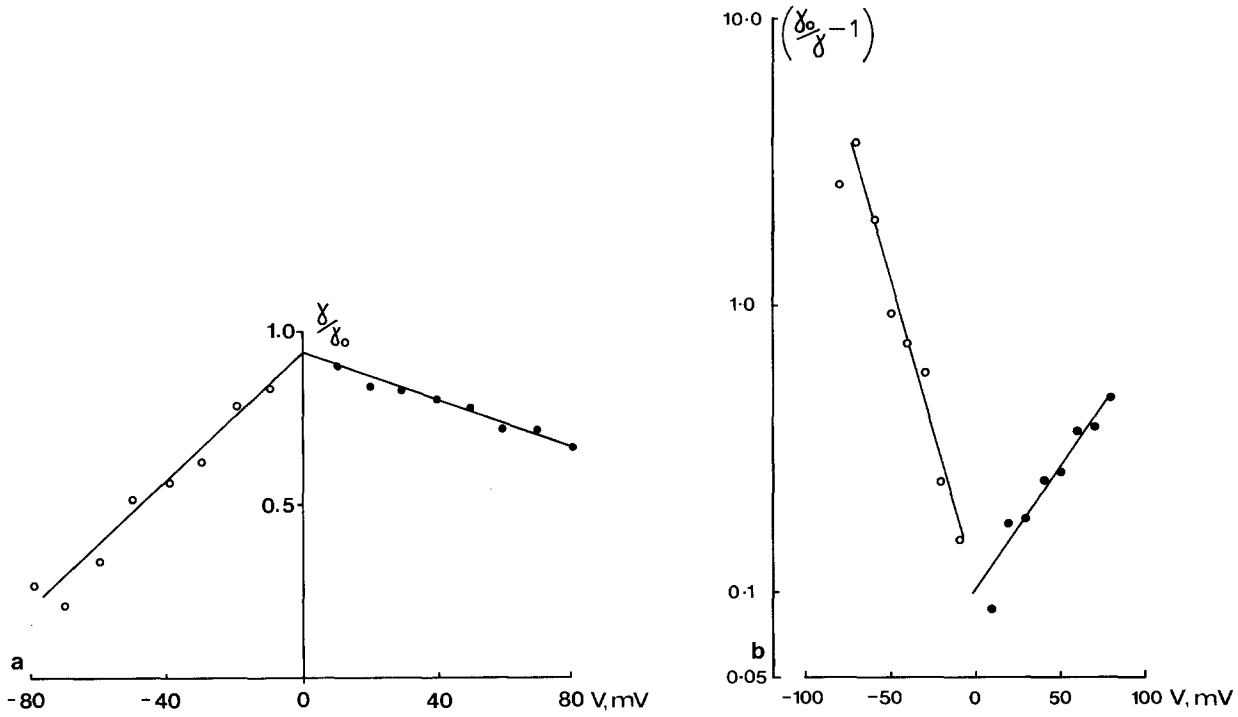

Fig. 6. Voltage dependence of decamethonium block. *a*) $\gamma/\gamma 0$ = the fraction of the total conductance remaining in the presence of the blocker (γ = reduced K^+ conductance in the presence of 200 μM decamethonium; $\gamma 0$ = full open-state conductance in the absence of blocker). The experiment was carried out with 75 mM K_2SO_4 solutions containing 200 μM decamethonium on both sides of the membrane. The recorded data was sampled every 8 msec and resolved to 256 conductance levels. Certain levels were designated to represent the full open state and all lower levels to represent the closed state (records with sub-state opening were not analyzed). The time averaged conductance was calculated for each 'flickering' opening by multiplying the fraction of the total number of samples in the open-state levels by the full open-state conductance, i.e. $\gamma/\gamma 0 \times \gamma 0$ = time averaged conductance. Each point represents a mean value of three experiments. Lines were drawn by least-squares regression. *b*) Linearized plot of Eq. (1) for the data displayed in Fig. 6*a*. Lines were drawn by least-squares regression

Discussion

Fractionation of mammalian cardiac muscle membrane vesicles as described by Jones and Cala (1981) yields three membrane populations of distinctly different densities. The lightest fraction (I) has been identified as being derived predominantly from the cardiac cell membrane (Jones et al., 1979). Fusion of fraction I vesicles with preformed planar phospholipid bilayers incorporates a number of species of channel (*data not shown*). Similar findings have recently been reported by Coronado and Latorre (1982). These channels are presumably involved in the normal excitable activity of the cardiac muscle cell (Noble, 1979). Membrane fractions II and III are subpopulations of the SR network. Following Ca^{2+} oxalate-loading Fraction III membrane vesicles pellet through 1.5 M sucrose due to the Ca^{2+} oxalate precipitated in the vesicle lumen. Fraction II vesicles accumulate little Ca^{2+} oxalate under normal loading conditions. However, this fraction may be stimulated to accumulate Ca^{2+} by ryanodine, 3×10^{-4} M ryanodine increasing Ca^{2+} uptake five- to 10-fold (Jones & Cala, 1981). The Ca^{2+}-accumulating abilities of fraction III vesicles are unaffected by ryanodine. In addition to their different Ca^{2+} handling capacities, the two populations display some differences in protein composition. Ryanodine-sensitive vesicles, in particular contain a 55-K dalton polypeptide, probably analogous to the calsequestrin molecule of skeletal muscle SR. Based on this evidence Jones and Cala suggested that the ryanodine-sensitive population of cardiac muscle SR (fraction II) originates from the terminal cisternae of the SR network. Our finding that both fractions II and III contain the same species of monovalent cation-selective channel may then be interpreted as suggesting that the channel is not localized to any specific region of the SR system.

Channel Substructure

The monovalent cation-selective channel of cardiac muscle SR appears to have one closed state and two

open states, designated here as α and β, with the α-state conductance being approximately 65% of the fully open β state. Multiple open states have now been described for a number of native membrane channels, including the voltage-dependent anion channel of the outer mitochondrial membrane (VDAC) (Colombini, 1979), the acetylcholine receptor (Hamill & Sakmann, 1981; Auerbach & Sachs, 1983), the chloride channel from *Torpedo* electroplax (Miller, 1982*b*; Hanke & Miller, 1983), the calcium-activated K^+ channel of rat skeletal muscle sarcolemma (Moczydlowski & Latorre, 1983) and the K^+ channel of the amphibian skeletal muscle SR channel (Labarca & Miller, 1981). The substructure of the channel described here differs somewhat from that of the amphibian skeletal muscle SR channel. The relative conductance of the two states is not the same, the α state of the amphibian channel being approximately 30% of the fully open β state (Labarca & Miller, 1981). The gating properties of the two channels are different. The amphibian skeletal muscle channel always enters the α state during transitions between the closed and the fully open, β state (Labarca & Miller, 1981). This is not the case for the mammalian cardiac muscle channel, where all possible transitions between the closed, α and β states are equally likely to occur.

What evidence do we have that the activity we report results from one multi-open-state channel rather than two independent two-state channels? We have never observed a fusion event that incorporated a channel showing just one of the open states. However, two possible combinations of two independent channels might explain the observed current fluctuations. One would involve one channel with an open-state conductance of 60 pS and another with an open-state conductance of 100 pS. Strongly countering this possibility is the fact that we have no records containing a single open state of 60 pS.

The second possible combination of two independent channels would involve channels with open-state conductances of 100 and 160 pS. For the commonly observed transition from a conductance of 100 to 160 pS to be explained by this combination of independent channels it would be necessary for the 160 pS channel to open simultaneously with the closing of the 100 pS channel. As the 100 to 160 pS transition is common it would be necessary for this event to have a reasonably high probability. This seems unlikely.

Further support for the multi-open-state channel is provided by our selectivity determinations. The monovalent cation selectivity sequence is identical for both open states. This is true for the sequence based on either conductance or permeability measurements (Table 1). The selectivity sequence described for the cardiac muscle SR channel is identical to those of both the mammalian skeletal muscle SR channel (Coronado et al., 1980) and the amphibian skeletal muscle SR channel (Labarca & Miller, 1981). As with these channels the permeability ratios for monovalent cations relative to K^+ are lower than the conductance ratios.

We have also observed qualitatively similar decamethonium-induced channel flickering from both open states to the same baseline, suggesting a common conductance pathway (Fig. 5*b*). This, together with the observations discussed above, leads us to conclude that the monovalent cation-selective channel of cardiac muscle SR is a multi-open-state channel.

Block by Cholinergic Drugs

Decamethonium will induce flickering of the cardiac muscle SR channel when added to either side of the planar bilayer. This is in contrast to the action of the compound on the mammalian skeletal muscle SR channel where it has been reported to act only from the *trans* side of the membrane (Coronado & Miller, 1980; Miller, 1982*a*). The possibility that we observe block from both sides of the membrane due to the insertion of the channel into the bilayer with variable orientation may be discounted. As with the mammalian skeletal muscle SR channel, the cardiac muscle SR channel always incorporates into the bilayer in the same orientation. This is readily demonstrated by observing the voltage dependence of the channel openings. In addition, the voltage dependence of the conduction block induced by decamethonium differs depending on whether the drug is acting from the *trans* or *cis* side of the membrane.

An inspection of the blocking parameters for decamethonium suggests that block of the cardiac muscle SR channel from the *trans* side of the bilayer closely resembles that reported for the mammalian skeletal muscle SR channel ($K(0) = 1.2$ mM, $z\delta = -1.22$ from Coronado and Miller, 1980). Based on the conclusions of Coronado and Miller (1980) and the subsequent studies of Miller (1982*a*) it would seem likely that decamethonium blocks monovalent cation conductance through the cardiac muscle SR channel from the *trans* side of the membrane by binding with high affinity to a site approximately 55% of the voltage drop across the channel from the *trans* side. This assumes that decamethonium binds in a bent configuration (Miller, 1982*a*) with z, the valence of the blocker = 2.

Decamethonium block of the cardiac muscle SR channel from the *cis* side of the bilayer shows a lower voltage dependence than block from the *trans* face of the membrane, and also has a slightly higher

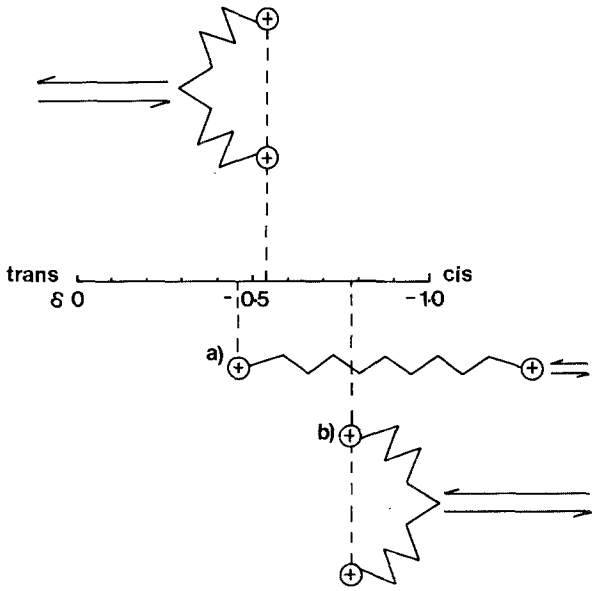

Fig. 7. Proposed mechanisms of decamethonium block from *trans* and *cis* face of the membrane. Decamethonium is shown binding in a bent centrifugation when entering the channel from the *trans* face of the membrane. This scheme is identical to that proposed by Coronado and Miller (1980) for decamethonium binding from the *trans* side of the membrane for the skeletal muscle SR channel. Two of the three proposed mechanisms for block from the *cis* side of the membrane are shown; *a*) with $z = 1$ and *b*) with $z = 2$. The third possibility is not shown, but would involve the inclusion of both quaternary ammonium groups of the molecule at different, as yet unidentified, sites within the voltage drop

zero voltage dissociation constant. These blocking parameters may be interpreted in three ways (Fig. 7). If decamethonium binds to the channel from the *cis* side of the bilayer in a bent configuration, with both positively charged quaternary ammonium groups at the same place within the voltage drop across the channel ($z = 2$), then our data indicate that the binding site for the blocker when added to the *cis* face of the membrane is 78% of the voltage drop within the channel from the *trans* face of the membrane. If decamethonium interacts with the channel from the *cis* side of the membrane in a linear conformation, so that only one quaternary ammonium group enters the voltage drop ($z = 1$), then the binding site for the blocker is only 46% of the voltage drop from the *trans* side of the membrane. Thirdly, if the decamethonium molecule were to enter the channel from the *cis* side of the bilayer in a linear configuration so that both charged groups are within the voltage drop but at different sites, as suggested by Miller for short chain bis *Qn* blockers from the *trans* face of the skeletal muscle SR channel (Miller, 1982*a*), then we can say nothing about the position of the binding sites within the voltage drop. Based on the evidence presented here it is not possible to conclusively state which of these three possibilities is correct for the *cis* block.

In conclusion, we have demonstrated the existence of a monovalent cation-selective channel in mammalian cardiac muscle SR. This channel appears to share a number of basic properties with the well-characterized monovalent cation-selective channel of skeletal muscle SR. It is probable that the channel described here is responsible for the recently reported macroscopic monovalent cation conductance of mammalian cardiac muscle SR (Meissner & McKinley, 1982) and as suggested by these authors, may fulfill a physiological role as a charge-compensating mechanism during electrogenic calcium fluxes associated with excitation-contraction coupling in the heart.

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