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Single Chloride-Selective Channel from Cardiac Sarcoplasmic Reticulum Studied in Planar Lipid Bilayers

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Summary. The behavior of single Cl^- channel was studied by fusing isolated canine cardiac sarcoplasmic reticulum (SR) vesicles into planar lipid bilayers. The channel exhibited unitary conductance of 55 pS (in 260 mM Cl^-) and steady-state activation. Subconductance states were observed. Open probability was dependent on holding potentials (−60 to +60 mV) and displayed a bell-shaped relationship, with probability values ranging from 0.2 to 0.8 with a maximum at −10 mV. Channel activity was irreversibly inhibited by DIDS, a stilbene derivative. Time analysis revealed the presence of one time constant for the full open state and three time constants for the closed states. The open and the longer closed time constants were found to be voltage dependent. The behavior of the channel was not affected by changing Ca^{2+} and Mg^{2+} concentrations in both chambers, nor by adding millimolar adenosine triphosphate, or by changing the pH from 7.4 to 6.8. The presence of sulfate anions decreased the unit current amplitude, but did not affect the open probability. These results reveal that at the unitary level the cardiac SR anion-selective channel has distinctive as well as similar electrical properties characteristic of other types of Cl^- channels.

Key Words chloride conductance · anionic permeability · intracellular membrane · sarcoplasmic reticulum · planar lipid bilayer

Introduction

In cardiac and skeletal muscle cells, the sarcoplasmic reticulum (SR) has been shown to be the major source and sink for Ca^{2+} (Endo, 1977). It is now well established that Ca^{2+} release from SR is the molecular event which triggers the contractile reaction. In order to study the physiological release mechanism, and the parameters possibly involved in its regulation, permeability properties of SR in cardiac skinned fibers (Fabiato, 1985) and isolated vesicles have been studied (Meissner & McKinley, 1982; Meissner & Henderson, 1987). The aim of these studies was to measure the SR membrane electrical properties and to identify the related protein constituents.

Recently, major progress has been made in understanding the molecular mechanisms controlling the SR Ca^{2+} release process. The activity of the SR Ca^{2+} release channel has been recorded using skeletal muscle (Smith, Coronado & Meissner, 1985, 1986) and cardiac SR Vesicles (Rousseau et al., 1986). Furthermore, the channel protein has been labeled with a specific probe, [3H]ryanodine, which allowed the isolation, characterization and reconstitution of the ryanodine receptor complex (Lai et al., 1988; Smith et al., 1988*b*).

The SR membrane contains additional ion-selective pathways besides the Ca^{2+} release channel. Monovalent cation and anion permeabilities have been demonstrated by ionic flux measurements using skeletal (Kometani & Kasai, 1978; McKinley & Meissner, 1978; Kasai, Kanemasa & Fukumoto, 1979) and cardiac SR vesicle fractions (Meissner & McKinley, 1982). More recently, the planar lipid bilayer-fusion technique coupled to voltage-clamp measurements (Miller & Racker, 1976), has been used to study K^+ and Cl^- conductances. In skeletal SR preparations, a K^+ channel has been studied in detail (Miller, 1978; Coronado, Rosenberg & Miller, 1980; Coronado & Miller, 1982; Garcia & Miller, 1984). Bretag (1987) has thoroughly reviewed the important role played by the muscle surface membrane chloride channels. However, little was known about the SR Cl^--selective channels at unitary level. Lately, two groups, using the bilayer-fusion technique, have made detailed observations concerning the single Cl^- channel present in mammalian skeletal muscle SR vesicles (Tanifuji, Sokabe & Kasai, 1987; Rousseau, Roberson & Meissner, 1988). Hals and Palade (1988) have also investigated the activity of a large conductance an-

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ion-selective channel using the patch-clamp technique, on the "sarcoball" preparation from frog skeletal muscles.

In this paper, I describe the functional properties of a single Cl^- channel present in canine cardiac SR vesicles. Multi- and single-channel recordings, ionic selectivity, voltage dependence, kinetic analysis, and inhibition by a stilbene derivative have been studied. The inhibition of the current by divalent anions and its insensitivity to divalent cations are also reported.

Materials and Methods

Chemical Reagents

Choline chloride and Trisma base (Tris) were obtained from Sigma (St. Louis, MO). HEPES and PIPES buffers were purchased from Research Organics (Cleveland, OH) and phospholipids from Avanti Polar Lipids (Birmingham, AL). Sulfuric acid, obtained from Fischer Scientific (Fair Lawn, NJ), was neutralized with Tris to produce stock solutions at pH 7.4. All the other materials were of reagent grade.

Preparation of Sarcoplasmic Reticulum Vesicles

Vesicles were prepared by differential and sucrose gradient centrifugation as described by Meissner and Henderson (1987). The crude microsomal fraction was further subdivided on a linear sucrose gradient 20–45% (wt/wt) using a Beckman Ti-14 zonal rotor. Five different fractions were recovered and assayed for their ability to induce single chloride channel activity.

Bilayer Formation and Vesicle Fusion

The bilayers were formed at room temperature from a lipid mixture containing phosphatidylethanolamine, phosphatidylserine purified from bovine brain, and diphytanoylphosphatidylcholine at a ratio of 5:3:2. The final lipid concentration was 25 mg/ml dissolved in decane. A 250-μm diameter hole, drilled in a polyvinyliden-difluoride (PVDF) cup, was pretreated with the same lipid mixture dissolved in chloroform. Using a Teflon stick, a drop of the lipid mixture in decane was gently spread across the bilarium hole in order to obtain formation of the planar artificial membrane. Membrane thinning was assayed by applying a triangle wave test pulse and typical capacitance values were in the range of 250–400 pF. Aliquots of vesicles (10–60 μg protein) were added to the *cis* chamber in the proximity of the bilayer. The fusion was either spontaneous or induced by stirring or applying negative holding potentials across the bilayer. To decrease the chance of additional vesicle fusions during an experiment, the free $[Ca^{2+}]$ of the *cis* chamber was lowered (<0.5 mM) by addition of EGTA or by perfusion with a HEPES-Tris buffer solution containing 1.2 μM free $[Ca^{2+}]$ (0.95 mM $CaCl_2$ and 1 mM EGTA).

Recording Instrumentation

Currents were recorded using a homemade double-stage amplifier (Smith et al., 1988*a*) or a DAGAN 8900; they were low-pass filtered (Frequency Device 902 LPF) and displayed on-line on a chart recorder (Z-1000, Astro Med.) and an oscilloscope (Nicolet 110). The currents were filtered (cut off frequency 4 kHz) and recorded on a video cassette recorder (Sony SL 2700) through a modified pulse code modulation device (Sony PCM 501ES) (Bezanilla, 1985). Current recordings were played back, filtered at 300 Hz and sampled at 1 kHz for storage on hard disk and for further analysis using an IBM PC-XT computer and programs kindly provided by Dr. H. Affolter. The open probability values (P_o) and time histograms were determined from data stored in 40-sec files, and the half-threshold discriminator method (Sachs, Neil & Bakakati, 1982) was used as previously described for time analysis of skeletal SR Cl^- channel in order to minimize the influence of subconducting states on chosen recordings (Rousseau et al., 1988).

Buffer Solutions

Cardiac as well as skeletal SR vesicles contain several types of ion channels, which conduct mono- and divalent cations and anions (Meissner & McKinley, 1982; Meissner, 1983). In order to record specific ionic currents, the composition of the chosen buffer should not contain permeant ions other than those of interest. Experimental conditions meeting this requirement have been worked out and described for observing skeletal SR Cl^- channel (Smith et al., 1988*a*; Rousseau et al., 1988). Briefly, cardiac SR vesicles were incorporated into planar liquid bilayers and Cl^- channels studied in asymmetric choline chloride (50 mM *trans*/250 mM *cis*), in the presence of 5 mM $CaCl_2$ and 10 mM HEPES, pH being adjusted to 7.4 with Tris. Choline, HEPES and Tris were considered impermeable for all practical purposes, so that chloride was the main permeant ion in the solution. The presence of symmetric millimolar calcium concentrations in the fusion buffer was required to stabilize the bilayer and promote vesicle fusions. Glass bi-distilled deionized water was used for preparing all buffer solutions. Different experimental conditions were obtained either by adding solubilized compounds directly to the chambers or by perfusing the entire volume of the chambers using a perfusion pump (Harvard Apparatus).

Table of Main Symbols Used

pS:	PicoSiemens
DIDS:	4,4'-Diisothiocyanostilbene 2,2'-disulfonic acid
EGTA:	[ethylenebis (oxyethylenenitrilo)] tetraacetic acid
HEPES:	N-2-Hydroxyethylenepiperazine-N'-2-ethanesulfonic acid
Tris:	Tris (hydroxymethyl) aminomethane
VDAC:	Voltage-dependent anion channels

Results

Channel Insertions

In the experiments reported herein, Cl^- channels were assayed by fusing canine cardiac SR vesicles

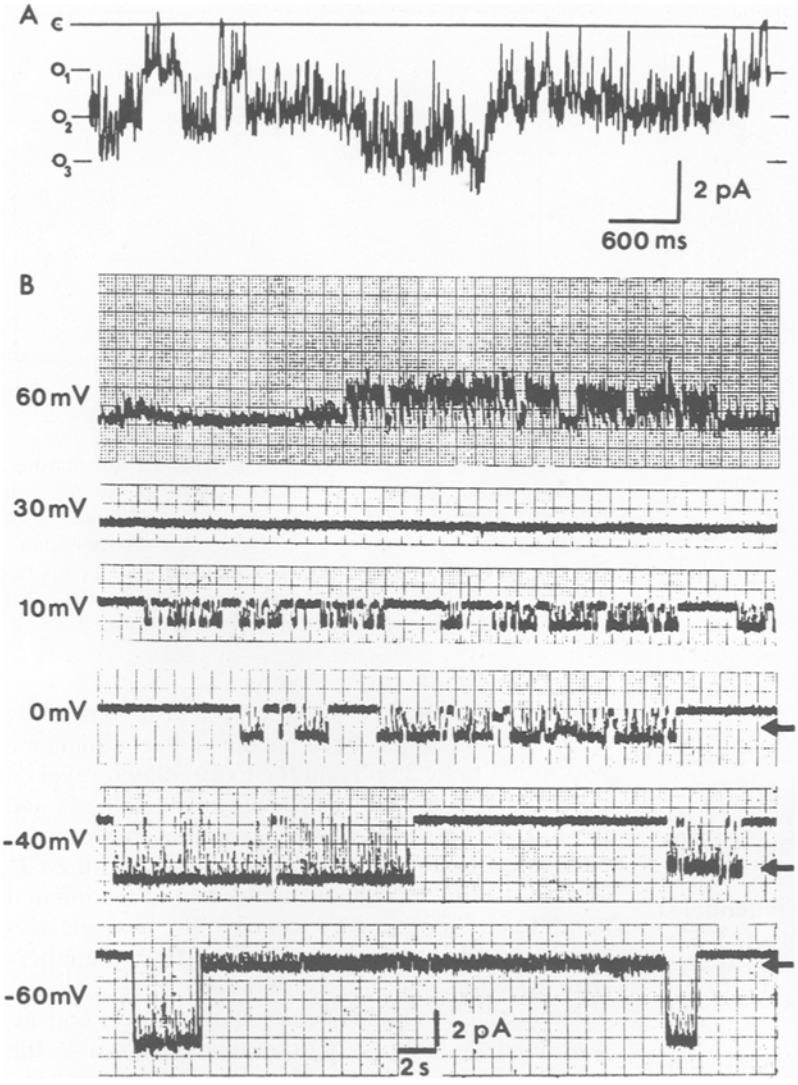

Fig. 1. Multi- and single-cardiac SR Cl^- channel recordings. Traces obtained in presence of 260 mM Cl^- *cis* and 60 mM Cl^- *trans*, pH 7.4. (*A*) At least three functional channels were present in the bilayer. Holding potential (HP) = 0 mV. The solid line represents the resting current level (*B*) Single-channel activity was recorded at different HPs (left). No currents were measured at +30 mV. *Cis* to *trans* Cl^- currents are shown as downward deflections. Arrows on the right correspond to the different subconductance levels

into planar lipid bilayers. Assuming that most of the vesicles were right side out—cytoplasmic side facing the bulk solution—it has been shown that after the fusion, the cytoplasmic side of the channel faces the *cis* compartment where the vesicles were added (Miller & Racker, 1976).

Spontaneous fusion occurred in the presence of the negatively charged phospholipid (phosphatidylserine) in the bilayer, of 5 mM Ca^{2+} in the aqueous phases and an osmotic gradient. Compared to the zero current baseline of the bare-layer, single vesicle fusion events were monitored by the appearance of discrete current steps (Tanifuji et al., 1987; Smith et al., 1988*a*). Typically, the incorporation of several anion-selective channels was observed in 80% of the fusions. In Fig. 1*A*, at least three distinct unit current levels can be observed. Single-channel recordings were obtained in 20% of the fusions (Fig. 1*B*). Following the first fusion event, further stepwise increases of the membrane conductance were obtained, but at a lower rate than that reported for skeletal SR vesicles (Tanifuji et al., 1987; Smith et al., 1988*a*). Consequently, in similar experimental conditions, cardiac SR vesicles are more difficult to fuse than those derived from skeletal SR preparations. On the other hand, the fact that step-size increases were of different amplitudes (*data not shown*) strongly supports the view that individual vesicles contain a various number (1–3) of Cl^- channels as already proposed for skeletal SR vesicles (Rousseau et al., 1988).

Over the five fractions recovered from the sucrose gradient, fractions III, IV and V, enriched in cardiac SR membranes (Meissner & Henderson, 1987) appeared to be positive in terms of Cl^- channel activity.

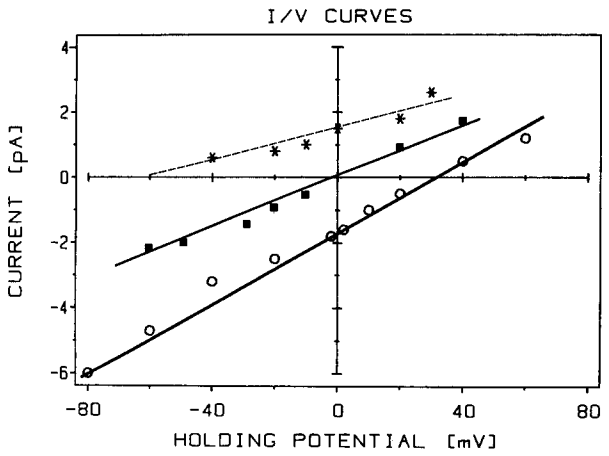

Fig. 2. Current-voltage relationship at different Cl^- concentrations. Circles: 260 mM *cis*/60 mM *trans*, γ = 55 pS; squares: 60 mM *cis*/60 mM *trans*, γ = 38 pS; and asterisks: 2.6 mM *cis*/60 mM *trans*, γ = 29 pS. For each curve, data points represent average values from nine, two and seven experiments, respectively

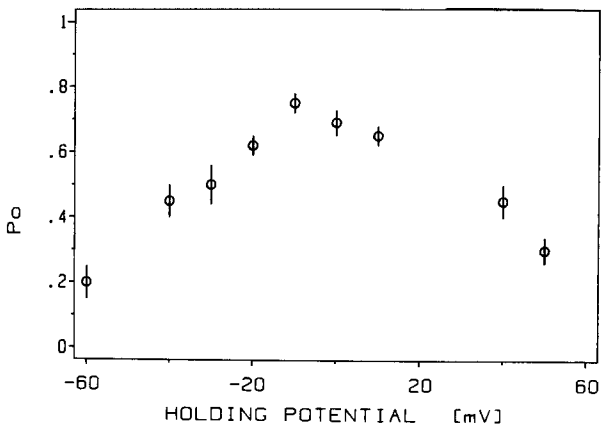

Fig. 3. Open probability *vs.* holding potential relationship. Steady-state P_o values were calculated from 40-sec files for each potential. Average values ± SD were calculated from five to seven experiments obtained in asymmetric Cl^- concentrations 260 mM *cis*/60 mM *trans*. Note the absence of data points between 10 and 40 mV; in this range, the unit current level was too small to allow an accurate determination of open events

Single-Channel Behavior

Figure 1*B* shows the current fluctuations of a single Cl^- channel recorded as a function of voltage in asymmetric Cl^- concentrations (260 mM Cl^- *cis*/60 mM Cl^- *trans*). Unit current amplitudes are directly proportional to the holding potential applied across the bilayer. In this example, the reversal potential is found at +30 mV where no activity is detected. For the other holding potentials, the channel displays a bursting behavior with an open probability, which is obviously voltage dependent.

The presence of subconducting states was another inference of these recordings (arrows; Fig. 1*B*). Typically, subconducting states are of short duration (<500 msec) but in some cases they are long lasting as shown for a holding potential of −60 mV (lower trace). In addition, other subconductance levels, ½ and ¾ of the full open state, were observed at 0 and −40 mV, respectively (Fig. 1*B*).

Figure 2 shows current-voltage relationships obtained with different Cl^- concentrations in the *cis* and *trans* chambers. In experiments performed in symmetric 60 mM [Cl^-], the unit conductance was 38 pS and the reversal potential was found at 0 ± 3 mV. In the presence of asymmetric Cl^- concentrations (260 mM Cl^- *cis* and 60 mM Cl^- *trans*), the unit conductance was 55 pS and the apparent reversal potential of +31 mV was close to the theoretical equilibrium potential of +36.7 mV calculated from the Nernst equation. After perfusion of the *cis* chamber with a Cl^--free buffer containing a higher density of HEPES/Tris, the Cl^- concentration was lowered to less than 2.6 mM (assuming a 99% removal of Cl^- ions). Consequently, with 60 mM Cl^- *trans* and <2.6 mM Cl^- *cis*, the unit conductance was found to be 29 pS and the extrapolated reversal potential was shifted toward negative values (−60 mV). The variations of the reversal potential with changing Cl^- concentrations demonstrated the Cl^- selectivity of the channel under our experimental conditions. Another way to prove the Cl^- selectivity of the channel was to perfuse both chambers with Cl^--free solution. This approach effectively eliminated Cl^- channel electrical activity, and allowed the observation of other channels such as the cardiac SR Ca^{2+} release channel (Rousseau et al., 1986; Rousseau, Smith & Meissner, 1987; Smith et al., 1988*a*). Furthermore, the reverse experiment can easily be performed: when the Cl^- electrical activity has been eliminated after perfusion with Cl^--free solutions, a typical Cl^- channel activity can be restored upon addition of 100 mM choline chloride in both or either chambers.

Voltage Dependence

In either asymmetrical or symmetrical Cl^- solutions, the cardiac SR anion channel displayed a significant voltage dependence. Figure 3 shows average steady-state open probability values as a function of the holding potential. A bell-shaped curve was obtained, with P_o values reaching a maximum of 0.8 at −10 mV and decreasing almost symmetrically for more positive and negative potentials. This behavior was very consistent from one recording to the other and was mainly due to the

Fig. 4. DIDS inhibition of the cardiac SR Cl⁻ channel. (*A*) In control conditions 260 mM Cl⁻ *cis*/60 mM Cl⁻ *trans* HP = 0 mV. Anions are moving across the channel under their chemical potential. (*B*) Upon addition of 10 mM DIDS and 4 sec after stirring (arrow). The channel activity displays a few full-size transitions and a period of low amplitude flickering behavior before complete and irreversible inhibition

presence of longer closed or inactivated states. A similar voltage-dependent relationship has been described for sarcolemmal Cl⁻ channels (Blatz & Magleby, 1986; Coulombe et al., 1987) and could be related to their only function as highly regulated charge carriers in biological membranes.

DIDS Inhibition

Stilbene derivatives are well known for inhibiting the anion transporter in red blood cells (Knauf & Rothstein, 1971). They are also potent inhibitors of anion permeability of skeletal SR vesicles (Kasai & Kometani, 1979; Yamamoto & Kasai, 1981). Half maximum inhibition of sulfate permeability was obtained in the presence of 0.06 mM DIDS (Kasai & Tagushi, 1981). SITS (2 mM) was used in single-channel experiments in order to inhibit anion-selective channels present in skeletal SR vesicles (Suarez-Isla et al., 1986). DIDS was also used at millimolar concentrations by Miller and White (1984) in order to abolish single Cl⁻ channel activity from *Torpedo* electroplax. In my hands, DIDS concentrations in the micromolar range had only a partial inhibitory effect on single Cl⁻ channel activity causing a 10 to 25% reduction of the opening probabilities. However, Fig. 4 shows that 10 mM DIDS completely inhibited cardiac SR Cl⁻ channel from the *cis* side in a few seconds, after a transition period where the channel gating and conducting properties were apparently modified. This effect was irreversible, since no recovery of activity was observed after extensive perfusion of the *cis* chamber with a DIDS-free choline chloride medium. Two other drugs known to bind specific, positively charged, amino-acid residues were used in order to probe the channel behavior. However phenylglyoxal (up to 10 mM) and diamine (up to 10 μM) had no detectable effect on cardiac SR Cl⁻ channel.

Channel Kinetics

Open and closed time constants were obtained by statistical analysis of the current fluctuations 30 sec after a new voltage was applied. For all the voltages studied using single-channel experiments, frequency histogram analysis consistently showed that the channel had essentially one main open state and multiple (three) closed states.

Figure 5 shows the cumulative open (upper panel) and closed (lower panel) time histograms. Note that the contribution of the longer closed events was eliminated from the closed time distribution by setting a maximum value for the longer classified events, (T_{max} = 250 msec) so that the present illustration (Fig. 5) corresponds to the intraburst analysis (Magleby & Pallota, 1983). Consequently, using single or double exponential fits (Barrett, Magleby & Palotta, 1982), only one open time constant and two closed time constants were found within bursts.

The open time constant (τop) and both closed time constants (τCl_1, and τCl_2) were determined at different voltages. Figure 6 (*A–B*) shows that the voltage dependence of the open time constant (τop) was the mirror image of that of the second closed time constant (τCl_2). Taking a limiting time resolution of 2 msec into account (Rousseau et al., 1988), the values of the shorter closed time constant (τCl_1) were essentially identical over the range of voltages studied. These data quantified and clarified the intraburst behavior of the channel. However, one must consider that a third closed time constant—a longer one—which was removed from the present analysis and corresponded to the interburst intervals was also voltage sensitive as previously mentioned during the visual examination of the current traces in Fig. 1*B*. Taken together, these results explain the voltage dependence of the open probability illustrated in Fig. 3.

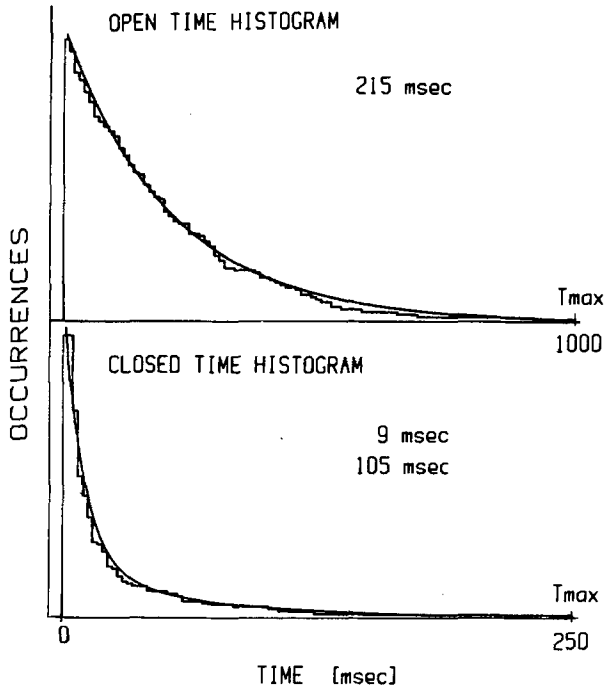

Fig. 5. Intraburst analysis in the form of cumulative open and closed time histograms for a cardiac SR Cl^- channel. A single channel was recorded as in Fig. 1*B*, HP = −10 mV. The open time distribution is described by only one exponential, fitted with a time constant of 215 msec (upper panel). The closed time distribution is described by the sum of two exponentials with time constants of 9 and 105 msec (lower panel)

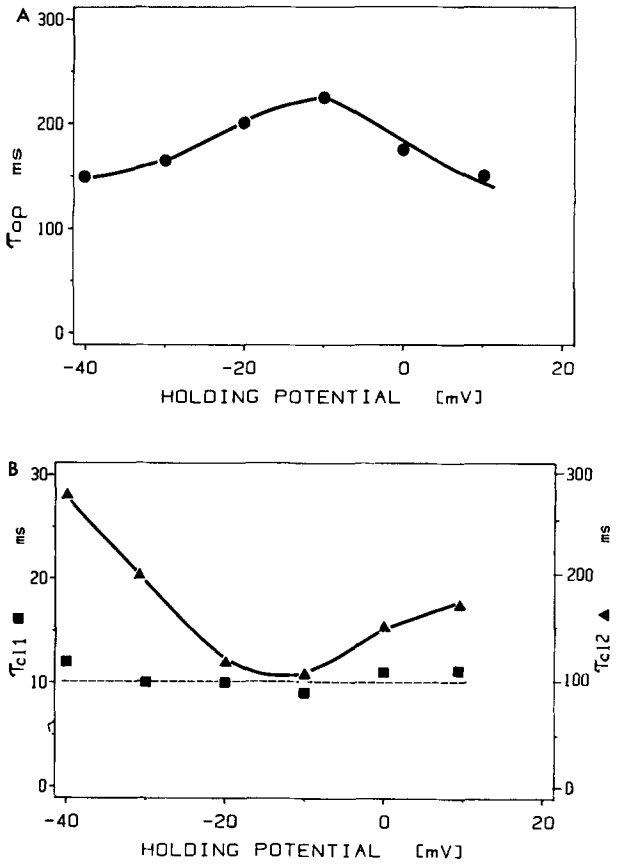

Fig. 6. Time constants for the open and closed states plotted as a function of the holding potential. (*A*) The open time constant (τop) has a maximum value at +10 mV, while the second closed time constant (τCl_2) presents a minimum value for the same voltage (*B*) Note that τCl_1 values are apparently voltage insensitive. Curves were drawn by eye

Effects of Divalent Ions

Flux measurements have indicated that divalent anion permeability is quite low through skeletal SR Cl^- channel (Kasai et al., 1979), while monovalent anions of the halide series are good charge carriers through Cl^- channels (Bormann, Hamill & Sakmann, 1987; Tanifuji et al., 1987). In our single-channel experiments, addition of sulfate anions (10–50 mM) in the *cis* chamber, induced a clear decrease in the unit Cl^- current amplitude for voltages more negative than the reversal potential, without any significant effect on the gating behavior (Figure 7). Identical results have previously been reported for the skeletal SR Cl^- channel (Tanifuji et al., 1987; Rousseau et al., 1988). It has also been noticed that sulfate ions are more potent blockers from the *cis* side than from the *trans* side.

Interestingly enough, under our experimental conditions, divalent cations such as Mg^{2+} and Ca^{2+} have no effect on the cardiac SR Cl^- channel activity. The channel's insensitivity to divalent cations was studied by varying the free Ca^{2+} (from 0.1 μM up to 50 mM) and Mg^{2+} concentrations (from 0.25 mM up to 2 mM) in both chambers. No detectable change in unit current amplitude, conductance, contribution of the subconducting states or opening probabilities was observed. Cardiac SR Cl^- channels were also insensitive to the presence and to the absence of adenine nucleotides, as well as to pH changes (7.4 to 6.8). A similar insensitivity to Ca^{2+}, Mg^{2+}, adenine nucleotide and pH has previously been reported for the anion-selective pathway present in skeletal SR vesicles (Rousseau et al., 1988).

Discussion

The SR anion permeability and its physiological regulation in skeletal and cardiac muscles are poorly understood. Herein, I have described the basic functional properties of a chloride-selective channel in canine cardiac SR vesicles.

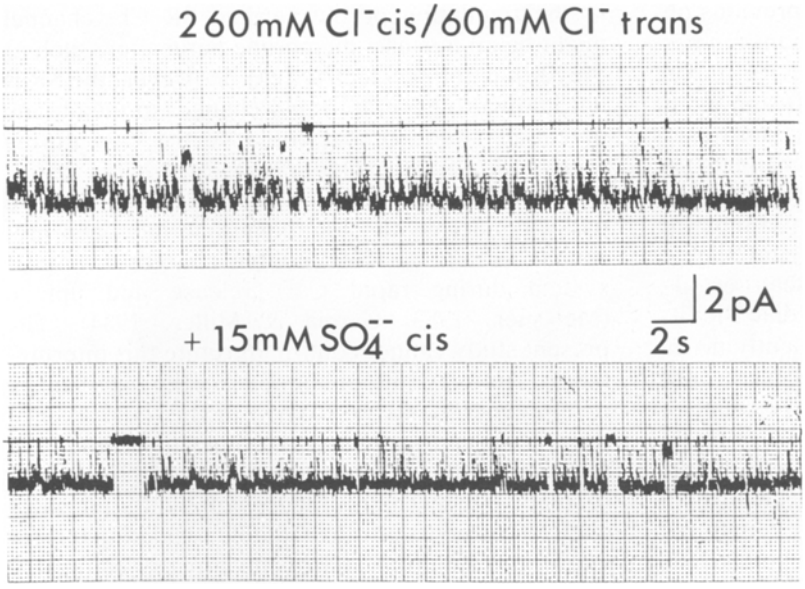

Fig. 7. Effect of sulfate anions on a single Cl^- channel in asymmetric choline chloride buffer. (*A*) Chart recording obtained in control conditions at −40mV. (*B*) Same conditions as in *A* but after addition of 15 mM $MgSO_4$ to the *cis* chamber. A reduction of the unit current amplitude can be observed. The solid lines represent the closed state levels

Considering the difficult task of studying the electrical properties of intracellular membranes, the planar lipid bilayer fusion technique provides a useful way for analyzing single-channel activities. This approach has allowed us to characterize K^+, (Miller, 1978; Coronado & Miller, 1982), and Ca^{2+} release channels, (Smith et al., 1985; Rousseau et al., 1986) and to test specific factors suspected of being involved in their regulation (Smith et al., 1986).

The Cl^--selective channel described in this paper shares several features with the skeletal SR Cl^- channel reported elsewhere (Tanifuji et al., 1987; Rousseau et al., 1988): presence of multiple subconducting states, similar gating behavior, stilbene derivative (DIDS) inhibition, divalent anion blockage and divalent cation insensitivity. Under identical experimental conditions, the cardiac SR Cl^- channel displayed, however, a lower unit conductance and steeper open probability *vs.* voltage relationship, than the mammalian skeletal SR Cl^- channel (Rousseau et al., 1988).

The presence of a Cl^- channel with a conductance similar to the one measured in the present study (55 pS) has previously been reported by Coronado and Latorre (1982) after fusion of calf cardiac "sarcolemma vesicles" into bilayers. The vesicle fractions used in the present study, although contaminated (20%) with sarcolemma membranes (Meissner & McKinley, 1982), have clearly been characterized, using enzyme-specific markers: Ca^{2+}-Mg^{2+}-ATPase activity, Ca^{2+} release channel and [3H]ryanodine binding, and are mainly derived from SR membranes (Meissner & Henderson, 1987). Furthermore, observing 55-pS Cl^- channels is a prerequisite for observing the characteristic cardiac Ca^{2+} release channels after perfusion with Cl^--free, Ca^{2+}-HEPES solutions. The simultaneous presence of Ca^{2+} release channels is probably the best evidence to state that the 55-pS Cl^- channel described herein is derived from SR membranes. On the other hand, only a large conductance (400 pS) Cl^- channel has been described at the surface membrane of cultured cardiac cells (Coulombe et al., 1987).

Another inference from our recordings is the presence of subconducting states, which have also been observed for many other Cl^- channels (Tank, Miller & Webb, 1982; Bormann et al., 1987; Miller & White, 1984; Nelson, Tang & Palmer, 1984; Schwarze & Kolb, 1984) suggesting that the existence of substates is a general characteristic of Cl^- channels. Their physiological relevance is not yet understood. Recently they have been referred to as the mean amplitudes of less frequently occurring elementary current steps (Bormann et al., 1987). Their common reversal potential with the main conducting state, for different Cl^- concentrations, argues in favor of their Cl^- selectivity.

It would be interesting to know the conductance of this channel under physiological conditions. Assumming an intracellular Cl^- activity of 7.2 ± 2.2 mM as measured by Caillé (1985) in rabbit papillary muscle, the unitary conductance would probably be less than 5 pS. In such conditions, the functional role of this channel may be linked to its gating properties and/or its distribution. Cardiac SR Cl^- channels from three different SR fractions, (III, IV and V as defined by Meissner & Henderson, 1987) have been incorporated into planar lipid bilay-

ers. This result is also consistent with previous observations that all these vesicle populations are permeable to Cl^- (Meissner & McKinley, 1982). The number of channels (1–3) per single fusion event was in the same range from trial to trial. This observation may support the idea that Cl^- channels are evenly distributed in the cardiac SR membrane. A similar result has been obtained when comparing the Cl^- channel distribution in "heavy" and "light" skeletal muscle SR fractions (Rousseau et al., 1988). The regulation process of the cardiac SR Cl^- channel remains to be established. Presently no intracellular gating or modulating agents have been found to interact with this anion-selective channel. Variations of SR transmembrane potential, or modifications of surface charge potentials might be responsible for the control of the gating mechanism in this channel. It has been shown that the open and closed time constants, (except τCl_1) are voltage dependent (Fig. 6). The following kinetic diagram is proposed to schematize the channel behavior:

$$O \rightleftharpoons C_1 \rightleftharpoons C_2 \rightleftharpoons C_3$$

where O represents the full open state and C_1, C_2 and C_3 the different closed states. Even if this pattern might also fit the one postulated for trout SR Cl^- channel (Rousseau et al., 1988) it would have to be completed since it does not take into account the presence of the subconducting states. However, the channel kinetic, conducting and opening probabilities do not match the properties of a dimeric Cl^- channel found in *Torpedo* electroplax surface membrane (Miller & White, 1984). Furthermore, the properties of the cardiac SR Cl^- channel are clearly different from those of the large conducting slowly gating (VDAC) channels derived from outer mitochondrial membrane (Colombini, 1979).

In agreement with result obtained by ionic flux and light scattering measurements on skeletal SR muscle vesicles (Kasai & Kometani, 1979), DIDS was found to be an inhibitor of this anion-selective channel. However, a more specific ligand with a higher affinity for this channel would be necessary in order to follow the channel protein through the different steps of a purification procedure.

Our experiments reveal that the unitary current from cardiac and skeletal SR Cl^- channels can be reduced by divalent anions. This blocking effect is certainly due to a competitive inhibition of the Cl^- current by sulfate ions. Furthermore, Tanifuji et al. (1987) have shown that this blocking effect was voltage dependent. The fact that large variations in divalent cation concentrations as well as the absence and the presence of adenine nucleotides have no effect, may suggest that the main factor involved in the regulation of the cardiac SR Cl^- channel might be an intrinsic membrane parameter, such as the SR membrane electric field. However, the SR is not known to generate rapid changes in membrane potential, although that could be the case during rapid Ca^{2+} release (Meissner & McKinley, 1982). The role of monovalent cation- and anion-selective pathways across the SR membrane has been presented as a plausible counter charges transport system during rapid Ca^{2+} release and uptake (Meissner, 1983; Garcia & Miller, 1984). The present study cannot confirm or refute this interpretation. Actually one might discuss the significance of the experiments performed in this study, since the present approach does not allow investigation of the cardiac SR Cl^- channel in its native lipid environment. Furthermore, the loss of membrane associate components or other soluble regulating factors during the isolation and fusion process cannot be ruled out. However, the technique used here remains the only one suitable for a direct observation of cardiac SR channels since no "sarcoball" preparation is available yet on this tissue.

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