Biophysical Journal Volume 67 December 1994 2280–2285

Streaming Potentials Reveal a Short Ryanodine-Sensitive Selectivity Filter in Cardiac Ca^{2+} Release Channel

Qiang Tu, Patricio Vélez, Malcolm Brodwick, and Michael Fill
Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77555-0641 USA

ABSTRACT Single cardiac sarcoplasmic reticulum Ca^{2+} release channels were reconstituted into planar bilayer membranes. Streaming potentials were measured in osmotically asymmetric solutions as a shift in the reversal potential. Potential changes induced by water movement through the bilayer (concentration polarization) and reduced ion activity in the concentrated nonelectrolyte solutions were determined using valinomycin. In 400 mM symmetrical $CsCH_3SO_3$, the average streaming potential was 2.74 ± 0.2 mV (n = 5, mean ± SE; 2 osmol/kg) and independent of the osmoticant used (sucrose or diglycine). Identical streaming potential magnitudes were obtained regardless of which side of the membrane the nonelectrolyte was placed. This suggests that the narrow part of the pore where single file diffusion occurs is relatively short (i.e., accommodates a minimum of 3 H_2O molecules). This value is comparable to similar measurements in a variety of surface membrane channels. Ryanodine-modified channels had no measurable streaming potential, an increased $Tris^+$ permeability relative to Cs^+, and decreased divalent selectivity (PCs/PTris 5.1 ± 1.1 to 1.7 ± 0.3, n = 3; PBa/PCs 8.2 ± 0.7 to 1.8 ± 0.5, n = 4). Cation/anion selectivity was essentially unaltered in ryanodine-modified channels. These results suggests that the narrow region of the permeation pathway (i.e., the selectivity filter) is relatively short and widens after ryanodine modification.

INTRODUCTION

The cardiac ryanodine receptor (RyR) is a large ligand gated Ca^{2+} channel. Hydropathy profiles and electron microscopic images indicate that >90% of its mass extends into the myoplasm (Nakai et al., 1990; Wagenknecht et al., 1989). The three-dimensional architecture deduced from electron micrographs suggests the RyR channel may have a long, narrow and branching permeation pathway (Wagenknecht et al., 1989). A long permeation pathway, however, would not be consistent with the large single channel conductance of isolated RyR channels in planar bilayers (gCa ≈ 80 pS, gK ≈ 600 pS; Smith et al., 1988). Typically, large conductance ion channels are thought to have relatively short pores (Latorre and Miller, 1983).

Despite its high conduction, the RyR is a Ca^{2+} selective ion channel (PCa/PK ≈7; Smith et al., 1988). Molecular sieving experiments suggest that the RyR permeation pathway narrows to a radius of ≈3.4 Å (Tinker and Williams, 1993). This region may be part of the channel's selectivity filter. In planar bilayers, RyR channel activity is commonly measured using Cs^+ as current carrier. If H_2O (radius 1.5 Å) and Cs^{2+} (radius 2 Å) pass through this narrow region of the channel in a single file fashion, then the length of this region can be estimated by streaming potential measurements. The magnitude of a streaming potential can be correlated to the number of H_2O molecules in the single file region (Rosenberg and Finkelstein, 1978; Levitt et al., 1978). A streaming potential ($V_{streaming}$) is defined as

$$V_{streaming} = N(\rho RT/F)\Delta II,$$

where N is the number of H_2O molecules in the single file region, ρ is the partial molar number of that water, R, T, and F have their usual meanings (and units), and ΔII is the osmotic pressure difference across the channel (osmol/kg). A channel which does not select between cations and anions or lacks a restricted, osmotically sensitive water space would not be expected to have a streaming potential ($V_{streaming}$ = 0).

In this study, we show that cardiac RyR channels have a significant streaming potential ($V_{streaming}$ = 2.74 mV). This suggests a single filing region which accommodates a minimum of three H_2O molecules. The $V_{streaming}$ measurement was independent of osmotic gradient direction and the chemical nature of the osmoticant. Ryanodine-modified channels had no streaming potential ($V_{streaming}$ ≈ 0), reduced $Cs^+/Tris^+$ permeability ratio and were less divalent selective. Since ryanodine-modified channels maintained ideal cation selectivity, loss of the streaming potential may be attributed to widening of the narrow region (i.e., single file assumption no longer valid). These data are consistent with the hypothesis that the RyR channel normally has a relatively short single filing compartment and that ryanodine modification physically widens that compartment.

MATERIALS AND METHODS

Sarcoplasmic reticulum microsome preparation

Heavy sarcoplasmic reticulum (SR) microsomes were prepared from dog cardiac muscle as previously described (Tate et al., 1985). Briefly, the left ventricles from dog hearts were diced and homogenized. SR microsomes were isolated by differential centrifugation and stored in 0.3 M sucrose, 0.9% NaCl, 10 mM Tris maleate, pH 6.8 at −80°C until used.

Received for publication 31 March 1994 and in final form 26 September 1994.

Address reprint requests to Dr. Michael Fill, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77555-0641. Tel.: 409-772-5469; Fax: 409-772-3381.

0006-3495/94/12/2280/06 $2.00

Channel incorporation and single channel recording

Planar lipid bilayers were formed across a 200-μm diameter aperture in the wall of a delrin (Small Parts Inc., Miami Lakes, FL) cup. Lipid bilayer-forming solution contained a 8:2 (by volume) mixture of phosphatdylethanolamine and phosphatidycholine (Avanti Polar Lipids, Pelham, AL) dissolved in decane at a final concentration of 50 mg/ml. SR vesicles were added in one side of the bilayer (defined as *cis*). The other side was defined as *trans* (virtual ground). Standard solutions contained 400 mM $CsCH_3SO_3$ *cis* (20 mM *trans*), 10 mM HEPES (pH 7.4), and 20 μM free Ca^{2+}. The free Ca^{2+} was measured using a Ca^{2+} electrode. After channel incorporation, the *trans* $CsCH_3SO_3$ was adjusted to 400 mM. The orientation of the incorporated channel is such that its cytoplasmic side is in the *cis* compartment, and its luminal side is in the *trans* compartment (Gyorke and Fill, 1993; Tu et al., 1994).

A custom current/voltage conversion amplifier was used to optimize single-channel recording (Hamilton et al., 1989). Acquisition software (pClamp: Axon Instruments, Burlingame, CA), an IBM-compatible 386 computer and a 12-bit analog-to-digital/digital-to-analog converter (Axon Instruments) were used. Single channel data were digitized at 5–10 kHz and filtered at 1 kHz.

Streaming potential measurements

Single channel current amplitudes were determined from amplitude histograms. Reversal potential (RP) was determined from linear regressions of current-voltage relationships. Once the channel was incorporated, a 2 osmol/kg osmotic gradient was established by adding nonpermeant nonelectrolytes (sucrose or diglycine) to one side of the bilayer (Meissner et al., 1989). Osmotic gradients induced a significant RP shift. This shift was attributed to three factors; 1) ion concentration polarization due to osmotic water movement across the bilayer, 2) altered ion activity in the nonelectrolyte solutions, and 3) presence of a streaming potential.

At the end of each experiment, valinomycin (25 nM) was added and the RP of the valinomycin-induced Cs^+ current determined. In some experiments, the RPs of single channel and valinomycin currents were actually measured simultaneously. Streaming potentials were determined as the difference between single channel and the valinomycin Cs^+ current RPs. Data are expressed as means ± SE.

Permeability measurements

Permeabilities of Ba^{2+} and $Tris^+$ ions relative to Cs^+ were determined for both normal and ryanodine-modified channels. Reversal potentials for normal or ryanodine-modified channel were determined by linear regression of current-voltage relationships in solution containing 200/400 $CsCH_3SO_3$ (*trans/cis*) and after 20 mM $Ba(CH_3SO_3)_2$ or 200 mM Tris CH_3SO_3 were added to the *trans* chamber. The relative permeability ratio of P_{Ba}/P_{Cs} or P_{Tris}/P_{Cs} was then calculated with ion concentrations and reversal potentials using the Goldman-Hodgdin-Katz relationship for mixtures of monovalent ions and the Meves-Vogel formulation (Meves and Vogel, 1973) for mixtures of divalent/monovalent ions.

RESULTS

Streaming potential experiments require that an osmotic gradient be applied across the channel. This was accomplished by adding a high concentration of nonpermeant nonelectrolyte to one side of the channel. However, considerable care must be taken to assure that streaming potential estimates are not influenced by certain unavoidable artifacts. First, the activity of ions in the concentrated nonelectrolyte solution will be reduced. Because the nonelectrolyte was added only to one side of the membrane, a transmembrane potential due to asymmetric ion activities or asymmetric junction potentials can arise. Second, the osmotic gradient across the bilayer will cause water movement through the membrane which can dilute ions on the opposite side. These local ion concentration changes can also induce a transmembrane potential. Rosenberg and Finkelstein (1978) devised a strategy using valinomycin to measure the magnitude of these membrane potential artifacts.

Valinomycin carries cations, but not water, across the membrane. Thus, valinomycin will not generate a streaming potential in the presence of an osmotic gradient (Levitt et al., 1978; Rosenberg and Finkelstein, 1978). Since the conductance and reversal potential of valinomycin is theoretically Nernstian, the valinomycin current-voltage relationship can be used to estimate the unavoidable membrane potential artifacts due to junction potentials, activity coefficients differences, and/or local dilution potentials.

In our RyR channel experiments, Cs^+ was used as current carrier. To determine whether valinomycin carries Cs^+ in a Nernstian fashion, the following control experiments were performed. In a 100/200 mM CsCl gradient (*cis/trans*), the reversal potential for valinomycin current was 12.9 mV but the Cs^+ equilibrium potential (E_{Cs}) was 18.1 mV. This difference can be attributed to osmotically induced concentration polarization. A distinctive feature of valinomycin is its high selectivity against Na^+ (Levitt et al., 1978), so NaCl was used to make the solutions isosmotic. After addition of 100 mM NaCl to the 100 mM CsCl solution, valinomycin Cs^+ current reversed at 17.9 mV, which is very close to E_{Cs}. Identical experiments done using $CsCH_3SO_3$ produced similar results. These observations confirm that valinomycin carries Cs^+ in a Nernstian fashion, effectively detects concentration polarization, and is highly selective for Cs^+ over Na^+ (Eisenman and Krasne, 1975).

To calculate the streaming potential generated by an osmotic gradient across open cardiac RyR channels, reversal potentials of RyR channel and valinomycin Cs^+ currents were measured under identical conditions. Sample single RyR channel currents recorded after 2 osmol/kg sucrose was added to one side (*trans*) of the bilayer are shown in Fig. 1 *A*. The records were acquired at different potentials in symmetrical 400 mM $CsCH_3SO_3$. At the theoretical E_{Cs} (0 mV), single channel currents were clearly resolved. The current-voltage relationship is shown in Fig. 1 *B*. Single RyR channel Cs^+ currents reversed at negative potentials (V_o; -14.5 ± 0.3 mV, $n = 5$, mean ± SE). Single channel conductance was 406 pS. Valinomycin-dependent Cs^+ current reversed at -11.8 ± 0.26 mV ($n = 5$; V_v, Fig. 1 *B* (inset)). The difference between V_o and V_v represents a streaming potential. Streaming potential magnitude ($V_o - V_v$) was 2.74 ± 0.2 mV ($n = 5$) in a 2 osmol nonelectrolyte gradient. When the osmotic gradient was reversed (sucrose applied to *cis* side), the streaming potential was comparable (3.15 ± 0.4 mV, $n = 3$). Further, identical values (3.01 ± 0.5 mV, $n = 3$) were obtained when the osmotic gradient was created by diglycine

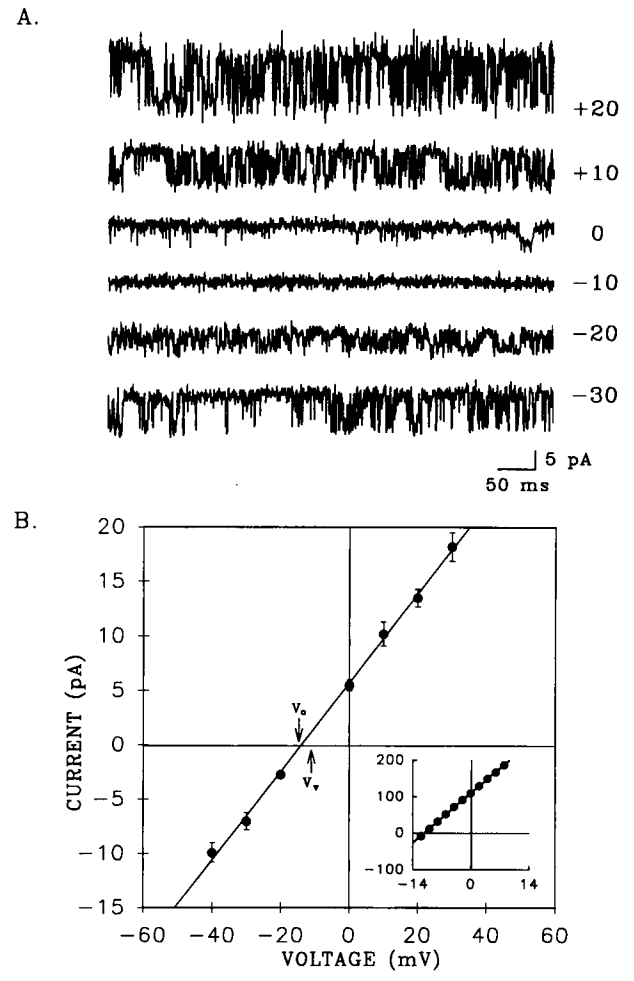

FIGURE 1 Streaming potential through a normal cardiac ryanodine receptor channel. Solutions contained symmetrical 400 mM $CsCH_3SO_3$ with 1 molal sucrose added to the *trans* side of the bilayer. (*A*) Single channel records at different membrane potentials. Potentials are labeled (as mV) in right margin. Closed current levels are marked by arrows. (*B*) Current-voltage relationship generated from several identical single channel experiments. Line is a linear regression. Points are means and error bars represent standard errors (no error bars indicate error is smaller than symbol size). Reversal potentials of RyR channel (V_o) and valinomycin Cs^+ currents (V_v) are marked. The streaming potential is the difference between these values ($V_o - V_v$). Valinomycin (25 nM) was applied symmetrically. The inset shows the current-voltage relationship of the valinomycin-dependent current.

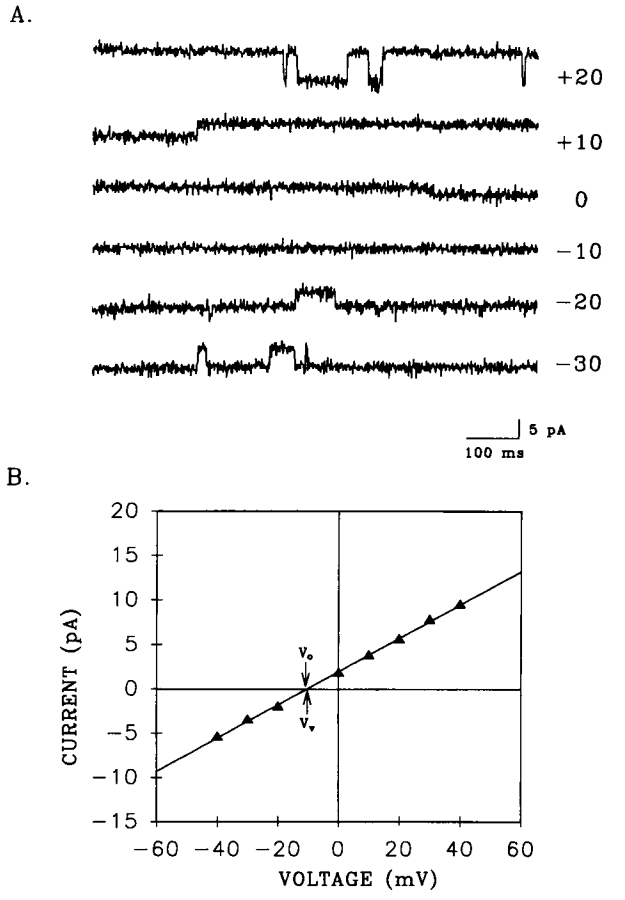

FIGURE 2 Streaming potential through a ryanodine-modified cardiac ryanodine receptor channel. Solutions contained symmetrical 400 mM $CsCH_3SO_3$ with sucrose added to the *trans* side of the bilayer. Ryanodine (2 μM) was applied to the *cis* chamber. (*A*) Single channel records at different membrane potentials. Potentials are labeled (as mV) in right margin. Closed current levels are marked by arrows. (*B*) Current-voltage relationship generated from several identical single channel experiments. Line is a linear regression. Points are means and error bars are smaller than the symbol size. Reversal potentials of RyR channel (V_o) and valinomycin Cs^+ currents (V_v) are marked. The streaming potential is the difference between these values ($V_o - V_v$). Valinomycin (25 nM) was applied symmetrically.

instead of sucrose. Thus, streaming potential magnitude was independent of osmotic gradient direction and the osmoticant used.

Ryanodine binding induces slow gating to a subconductance level (Smith et al., 1988). Streaming potential measurements were also performed on ryanodine-modified channels. Currents through ryanodine-modified channels recorded in an osmotic gradient (2 osmol/kg sucrose; *trans*) are shown in Fig. 2 *A*. Records were obtained at different potentials in symmetrical 400 mM $CsCH_3SO_3$. Again, single channel currents were resolved at the theoretical E_{Cs} (0 mV). The current-voltage relationship (gCs^+ = 187 pS) is shown in Fig. 2 *B*. The reversal potentials of single channel Cs^+ current (V_o) and valinomycin-dependent Cs^+ current (V_v) were identical. Thus, there was no streaming potential ($V_o - V_v = 0.06 \pm 0.54$; $n = 7$) in ryanodine-modified channels.

Loss of the streaming potential in ryanodine-modified channels could reflect loss of anion-cation selectivity where the osmotic gradient could push both cations and anions through the channel (i.e., no net streaming potential). If this were the case, ryanodine-modified channels would become equally permeable to Cs^+ and $CH_3SO_3^-$ (i.e., $PCs/PCH_3SO_3 = 1$). In Fig. 3, the current-voltage relationships of normal and ryanodine-modified channels measured in a 200/400 mM $CsCH_3SO_3$ (*trans/cis*) gradient are shown. Normal RyR channel current reversed at -20.3 ± 0.20 mV ($n = 3$). Ryanodine-modified channel current reversed at -20.0 ± 0.43 mV ($n = 3$). The nearly identical reversal potentials indicate that no significant difference ($P > 0.8$; two-sided *t*-test) in ion selectivity exists. Thus, the loss of streaming

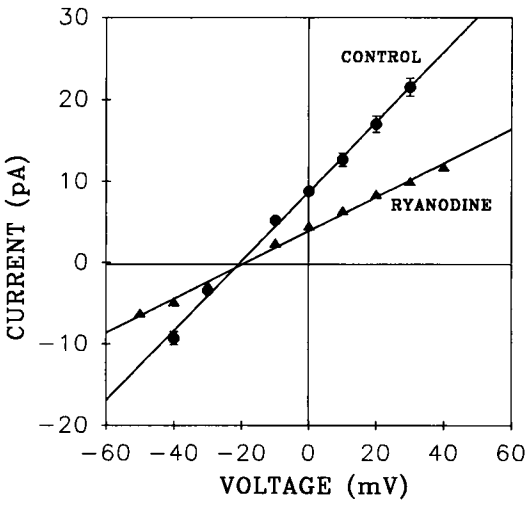

FIGURE 3 Current-voltage relationships in a 200/400 mM $CsCH_3SO_3$ gradient. Circles represent data collected on several normal RyR channels. Triangles represent data collected on several ryanodine-modified channels. Data points are means and error bars represent standard errors (no error bars indicate error is smaller than symbol size). Lines are linear regressions.

potential in ryanodine-modified channels cannot be explained by loss of cation/anion selectivity.

Our demonstration of a streaming potential in normal RyR channels and a published estimate of the pore size (radius ≈3.4; Tinker and Williams, 1993), suggest that H_2O and Cs^+ may diffuse in single file fashion through a narrow region in the permeation pathway. It is possible that ryanodine modification widens this narrow region so that single filing no longer occurs. If true, then the permeability of ryanodine-modified channels to large cations may increase. In Fig. 4 *A*, the relative permeability of Cs^+ and $Tris^+$ through normal (circles) and ryanodine-modified (triangles) RyR channels was determined from reversal potential shifts. Current-voltage relationships were acquired in a 400/200 mM $CsCH_3SO_3$ gradient (*cis/trans*) after 200 mM $TrisCH_3SO_3$ (*trans*) was added. After the addition of $TrisCH_3SO_3$, current through the normal RyR channel reversed at -14.9 ± 2.1 mV ($n = 3$) and current through the ryanodine-modified channel reversed at -4.4 ± 1.6 mV ($n = 3$). Dotted line represents current-voltage relationship before addition of the Tris-CH_3SO_3 (E_{Cs} marked). The PCs/PTris$^+$ was 5.1 ± 1.1 ($n = 3$) in normal and 1.7 ± 0.3 ($n = 3$) in ryanodine-modified channels. This significant ($P < 0.02$) increase in $Tris^+$ permeability is consistent with the hypothesis that ryanodine-modified channels have a wider pore.

In Fig. 4 *B*, the relative permeability of Ba^{2+} and Cs^+ through normal (circles) and ryanodine-modified (triangles) RyR channels was determined. Current-voltage relationships were acquired in a 400/200 mM $CsCH_3SO_3$ gradient (*cis/trans*) after 20 mM $Ba(CH_3SO_3)_2$ (*trans*) was added. After the addition of Ba^{2+}, current through the normal RyR channel reversed at 7.8 ± 1.2 mV ($n = 4$) and current through the ryanodine-modified channel reversed at -8.7 ± 1.1 mV ($n = 3$). The PBa/PCs$^+$ was 8.2 ± 0.7 ($n = 4$) in normal and 1.8 ± 0.5 ($n = 3$) in ryanodine-modified channels.

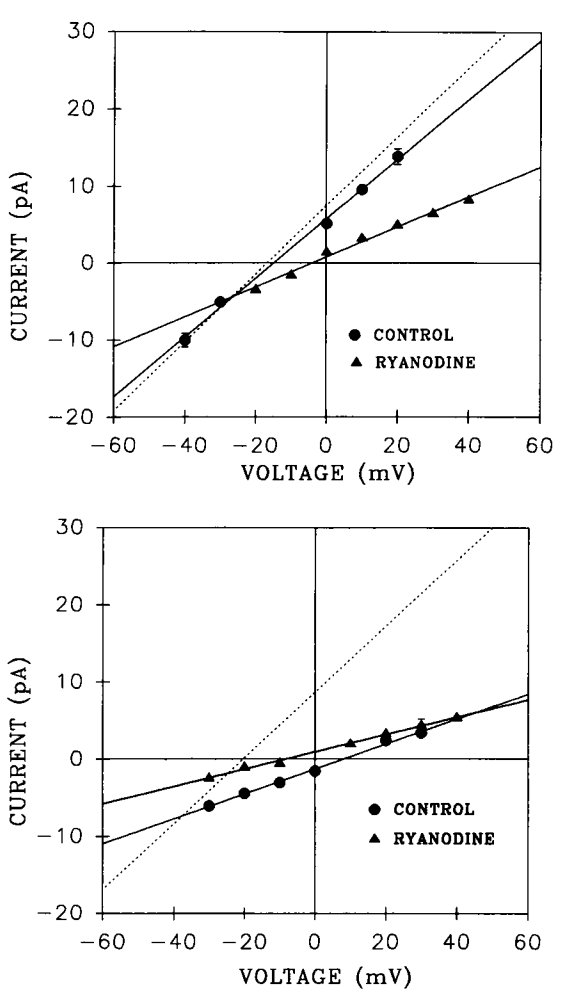

FIGURE 4 Current-voltage relationships demonstrating $Tris^+$ and Ba^{2+} permeability relative to Cs^+. Control solution contained 400 mM (*cis*) and 200 mM (*trans*) $CsCH_3SO_3$. Dotted lines represents data collected from normal channels in the control solution. Data points (means ± SE) represent several normal (circles) and ryanodine-modified (triangles; 2 μM ryanodine) channels in presence of a competing ion. (*A*) After 200 mM $TrisCH_3SO_3$ was added to the control solution in the *trans* chamber. Single channel current reversed at −14.9 and −4.4 mV for the normal and ryanodine-modified channels respectively. (*B*) After 20 mM $Ba(CH_3SO_3)_2$ added to the control solution in the *trans* chamber. Single channel currents reversed at 7.8 and −8.7 mV for the normal and ryanodine-modified channels, respectively.

Thus, ryanodine modification also induced a significant ($P < 0.001$) decrease in Ba^+ selectivity.

DISCUSSION

Streaming potentials were observed as relatively small osmotically induced shifts in reversal potential. Therefore, any factor which can potentially shift the current-voltage relationship can influence the streaming potential measurement (Rosenberg and Finklestein, 1978; Barry and Diamond, 1984). Possible sources of error include junction potentials, osmotically induced concentration polarization and altered ion activities in the concentrated nonelectrolyte solution. To

eliminate these and other potential sources of error, the valinomycin strategy devised by Rosenberg and Finklestein (1978) was employed. The reversal of valinomycin Cs^+ current was used to determine the actual E_{Cs} in the presence of the osmotic gradient with all the conceivable sources of error present. Thus, the difference between single RyR channel and valinomycin Cs^+ current reversal potentials was used as a reasonable measure of the streaming potential.

One possible caveat is that the reversal potential shift, we interpret as a streaming potential, could be due to a pharmacological action of the osmotic effector. For example, sucrose may alter channel properties (e.g., selectivity). Identical streaming potentials estimates, however, were obtained using pharmacologically different osmotic effectors (sucrose and diglycine). Further, the streaming potential estimate was independent of osmotic gradient direction implying that the osmotic effectors had identical action from both sides of the channel.

The apparent number of H_2O molecules in the single file region of the channel was estimated. A streaming potential of 2.74 mV suggests the channel contains a minimum of three H_2O molecules. Three H_2Os (radius 1.5 Å each) would cover a distance of 9 Å. Examples of streaming potential measurements (corrected to 2 osmol/kg) in other ion channels are 1.7 mV (K_{Ca} channel, 2 H_2O; Alcayaga et al., 1989), 6.0 mV (gramicidin A, 7 H_2O; Rosenberg and Finkelstein, 1978), 2.2 mV (SR K^+ channel, 3 H_2O; Miller, 1982), and 2.4 mV (hemocyanin channel, 3 H_2O; Cecchi et al., 1982). Thus, the RyR channel does not have an unusually large streaming potential. This implies that the narrow region is relatively short. This is consistent with the large conductance of the RyR channel (Latorre and Miller, 1983). We note that these estimates are minima based on the assumption of a single file compartment. If this assumption is relaxed, the equivalent length becomes longer. The existence of a single filing region in the normal RyR channel, however, is independently supported by permeability studies (Tinker and Williams, 1993).

A three-dimensional reconstruction deduced from electron micrographs suggests the RyR channels have long branching tunnels (>15 nm) which may represent the permeation pathway (Wagenknecht et al., 1989). Higher resolution imaging has revised the original channel architecture and now suggest a shorter (≈8 nm) straight transmembrane pore (Radermacher et al., 1994). Modeling of ion permeation through the cardiac RyR channel suggest the selectivity filter is in the electric field of the membrane (Tinker and Williams, 1993). Combined with these results our data suggests that the narrow single file region is part of the selectivity filter which is in the plane of the electric field and may represent a small part of a long permeation pathway.

The loss of the streaming potential in ryanodine-modified channels was not due to loss of cation-anion selectivity. Since ryanodine increased $Tris^+$ permeability and decreased Ba^{2+} selectivity of the RyR channel, we propose that ryanodine binding widens a narrow region which is probably part of the channel's selectivity filter. This is consistent with recent permeation data which suggest that ryanodine alters the structure of the conduction pathway (Lindsay et al., 1994). The simultaneous alteration of channel gating and ion selectivity is not without precedent. Aconitine decreases cationic discrimination while changing Na^+ channel kinetics (Campbell, 1982).

If ryanodine binding widens the pore, then why do ryanodine-modified channels have smaller conductance? One possibility is that ryanodine increases the energy barriers for ion permeation. For example, ryanodine might reduce the effective capture radius of the channel while maintaining enough space for H_2O and Cs^+ to freely pass each other (i.e., no single filing). Experiments with quaternary amine blockade of RyR channels support this possibility (Tinker and Williams, 1993). It is also possible that ryanodine deepens energy wells associated with a cation binding site in the permeation pathway (i.e., jumping rates out of the wells would be slower reducing the conductance). Thus, our suggestion that ryanodine widens a narrow region in the pore is not, in principle, incompatible with reduced conduction.

Although the assumption that a single filing region exists in the RyR channel's conductive pathway is supported by selectivity and pore size measurements (Smith et al., 1988; Tinker and Williams, 1993), it is possible that single filing does not occur. Streaming potentials can also be generated through artificial wide pores (e.g., capillary tubes) and are thought to be due to cation flux coupling to solvent flow by a frictional mechanism (Levitt et al., 1978; Miller, 1982). If this occurs in the RyR channel, interpretation of V_s magnitude would be impossible without independent estimation of several thermodynamic parameters (Levitt et al., 1978; Miller, 1982). For such a wide pore model, the flux coupling would critically depend on the charge structure of the pore. For example, if ions were tightly bound to the walls, the slippage of water molecules around them would result in little solute drag. If the RyR channel is best described by such a wide pore model, then ryanodine would have to alter the charge structure of the pore in order to simultaneously reduce selectivity, channel conductance, and streaming potential.

SUMMARY

In summary, the RyR channel's permeation pathway contains a narrow region in which diffusion of H_2O and Cs^+ may be single file. Streaming potential measurements reveal that the length of this narrow region is short and is similar to values obtained in a variety of other channels. Ryanodine-modified channels had no streaming potential, increased $Tris^+$ permeability, decreased Ba^{2+} selectivity, but retained cation-anion selectivity. This supports the hypothesis that ryanodine widens a narrow region in the RyR channel's selectivity filter.

This work was supported by National Institutes of Health grant R29-AR41197 (M.F.). Michael Fill is an Established Investigator of the American Heart Association.

REFERENCES

Alcayaga, C., X. Cecchi, O. Alvarez, and R. Latorre. 1989. Streaming potential measurements in Ca^{2+}-activated K^+ channels from skeletal and smooth muscle. *Biophys. J.* 55:367–371.

Barry, P. H., and J. Diamond. 1984. Effects of unstirred layers on membrane phenomena. *Physiol. Rev.* 64:761–872.

Campbell, D. T. 1982. Modified kinetics and selectivity of sodium channels in frog skeletal muscle fibers treated with aconitine. *J. Gen. Physiol.* 80:713–731.

Cecchi, X., R. Bull, R. Franzoy, R. Coronado, and O. Alvarez. 1982. Probing the pore size of the hemocyanin channel. *Biochim. Biophys. Acta.* 693: 173–176.

Eisenman G., and S. J. Krasne. 1975. The ion selectivity of carrier molecules, membranes and enzymes. *In* MTP International Review of Science, Biochemistry Series, Vol. 2. C. F. Fox, editor. Butterworths, London. 27–59.

Gyorke, S., and M. Fill. 1993. Ryanodine receptor adaptation: control mechanism of Ca^{2+}-induced Ca^{2+} release in heart. *Science.* 260:807–809.

Hamilton, S. L., R. M. Alvarez, M. Fill, M. J. Hawkes, K. L. Brush, W. P. Schilling, and E. Stefani. 1989. [^{3}H]PN200–110 and [^{3}H]ryanodine binding and reconstitution of ion channel activity with skeletal muscle membrane. *Anal. Biochem.* 183:31–41.

Latorre, R., and C. Miller. 1983. Conduction and selectivity in potassium channels. *J. Membrane Biol.* 71:11–30.

Levitt, D. G., S. R. Elias, and J. Hautman. 1978. Number of water molecules coupled to the transport of sodium potassium and hydrogen ions via gramicidin, nonactin or valinomycin. *Biochim. Biophys. Acta.* 512: 436–451.

Lindsay, A. R. G., A. Tinker, and A. J. Williams. 1994. Ion conduction in the ryanodine-modified SR Ca^{2+} release channel. *Biophys. J.* 66:314a.

Meissner, G., E. Rousseau, and A. Lai. 1989. Structural and functional correlation of the trypsin-digested Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* 264:1715–1722.

Meves, H., and W. Vogel. 1973. Calcium inward currents in internally giant axons. *J. Physiol.* 235:225–265.

Miller, C. 1982. Coupling of water and ion fluxes in a K^+-selective channel of sarcoplasmic reticulum. *Biophys. J.* 38:227–230.

Nakai, J., T. Imagawa, Y. Hakamata, M. Shigekawa, H. Takeshima, and S. Numa. 1990. Primary structure and functional expression from cDNA of the cardiac ryanodine receptor/calcium release channel. *FEBS Lett.* 271: 169–177.

Radermacher M., V. Rao, T. Wagenknecht, R. Grassucci, J. Frank, A. P. Timerman, and S. Fleischer. 1994. Three-dimensional cryo-electron microscopy of the calcium release channel/ryanodine receptor from skeletal muscle. *Biophys. J.* 66:19a.

Rosenberg, P. A., and A. Finkelstein. 1978. Interactions of ions and water in gramicidin A channels. Streaming potentials across lipid bilayer membrane. *J. Gen. Physiol.* 72:327–340.

Smith, J. S., T. Imagawa, J. Ma, M. Fill, K. P. Campbell, and R. Coronado. 1988. Purification ryanodine receptor from rabbit skeletal muscle is the calcium-release channel of sarcoplasmic reticulum. *J. Gen. Physiol.* 92: 1–26.

Tate, C. A., R. J. Bick, A. Chu, W. B. Van Winkle, and M. L. Entman. 1985. Nucleotide specificity of cardiac sarcoplasmic reticulum. GTP-induced calcium accumulation and GTPase activity. *J. Biol. Chem.* 260: 9618–9623.

Tinker A., and A. J. Williams. 1993. Probing the structure of the conduction pathway of the sheep cardiac sarcoplasmic reticulum calcium-release channel with permeant and impermeant organic cations. *J. Gen. Physiol.* 102:1107–1129.

Tu, Q., P. Velez, M. C. Gutierrez, and M. Fill. 1994. Surface charge potentiates conduction through the cardiac ryanodine receptor channel. *J. Gen. Physiol.* 103:853–867.

Wagenknecht, T., R. Grassucci, J. Frank, A. Saito, M. Inui, and S. Fleischer. 1989. Three-dimensional architecture of the calcium channel/foot structure of sarcoplasmic reticulum. *Nature.* 338:160–170.