

How Does Ryanodine Modify Ion Handling in the Sheep Cardiac Sarcoplasmic Reticulum Ca^{2+} -Release Channel?

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ABSTRACT Under appropriate conditions, the interaction of the plant alkaloid ryanodine with a single cardiac sarcoplasmic reticulum Ca^{2+} -release channel results in a profound modification of both channel gating and conduction. On modification, the channel undergoes a dramatic increase in open probability and a change in single-channel conductance. In this paper we aim to provide a mechanistic framework for the interpretation of the altered conductance seen after ryanodine binding to the channel protein. To do this we have characterized single-channel conductance with representative members of three classes of permeant cation; group 1a monovalent cations, alkaline earth divalent cations, and organic monovalent cations. We have quantified the change in single-channel conductance induced by ryanodine and have expressed this as a fraction of conductance in the absence of ryanodine. Fractional conductance seen in symmetrical 210 mM solutions is not fixed but varies with the nature of the permeant cation. The group 1a monovalent cations (K^+ , Na^+ , Cs^+ , Li^+) have values of fractional conductance in a narrow range (0.60–0.66). With divalent cations fractional conductance is considerably lower (Ba^{2+} , 0.22 and Sr^{2+} , 0.28), whereas values of fractional conductance vary considerably with the organic monovalent cations (ammonia 0.66, ethylamine 0.76, propylamine 0.65, diethanolamine 0.92, diethylamine 1.2). To establish the mechanisms governing these differences, we have monitored the affinity of the conduction pathway for, and the relative permeability of, representative cations in the ryanodine-modified channel. These parameters have been compared with those obtained in previous studies from this laboratory using the channel in the absence of ryanodine and have been modeled by modifying our existing single-ion, four-barrier three-well rate theory model of conduction in the unmodified channel. Our findings indicate that the high affinity, essentially irreversible, interaction of ryanodine with the cardiac sarcoplasmic reticulum Ca^{2+} -release channel produces a conformational

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alteration of the protein which results in modified ion handling. We suggest that, on modification, the affinity of the channel for the group 1a monovalent cations is increased while the relative permeability of this class of cations remains essentially unaltered. The affinity of the conduction pathway for the alkaline earth divalent cations is also increased, however the relative permeability of this class of cations is reduced compared to the unmodified channel. The influence of modification on the handling by the channel of the organic monovalent cations is determined by both the size and the nature of the cation. Small cations such as ammonia respond to ryanodine-induced alterations of the conduction pathway in much the same way as the group 1a monovalents. Larger organic cations such as diethanolamine and diethylamine have an increased relative permeability following ryanodine modification. Conductance of these cations is also influenced by the relative importance of their interactions with both hydrophobic and hydrophilic binding sites of the conduction pathway. These altered parameters of ion handling have been incorporated into an Eyring rate theory model and provide an adequate description of the observed variation in fractional conductance seen on modification of the channel by ryanodine in the presence of the different classes of permeant cation.

INTRODUCTION

Ryanodine is a plant alkaloid which disrupts cardiac muscle function (Sutko, Willerson, Templeton, Jones, and Besch, 1979; Sutko and Willerson, 1980) through an interaction with the release mechanism of the major intracellular Ca^{2+} store, the sarcoplasmic reticulum (SR) (Sutko, Ito, and Kenyon, 1985) and in recent years, has been widely used as a tool in the elucidation of the role of the SR in cardiac muscle excitation-contraction coupling (Marban and Wier, 1985; Beuckelmann and Wier, 1988; Bers and Bridge, 1989; DuBell, Lewartowski, Spurgeon, Silverman, and Lakatta, 1993; Negretti, O'Neill, and Eisner, 1993). The high affinity, essentially irreversible, binding of [^3H]-ryanodine to the SR Ca^{2+} -release channel protein has made possible the purification of this receptor and its characterization as a ligand-regulated, cation-selective ion channel (Lai, Erickson, Rousseau, Liu, and Meissner, 1988a; Lai, Erickson, Rousseau, Liu, and Meissner, 1988b; Lindsay and Williams, 1991). At the single-channel level, ryanodine has been reported to produce a number of concentration-dependent alterations of channel function. Broadly, low concentrations (nanomolar) are reported to increase single-channel open probability (P_o), while high concentrations (millimolar) lead to an abolition of channel opening ($P_o = 0$). Intermediate concentrations of ryanodine produce profound modifications of channel gating and conduction which are manifest as the occurrence of a reduced conductance, high open probability, state (Rousseau, Smith, and Meissner, 1987; Ashley and Williams, 1990). Residence in this ryanodine-modified state is, on the timescale of single-channel experiments, irreversible; removal of ryanodine from the medium bathing the channel protein does not bring about the return of normal channel gating and conduction. Attempts have been made to correlate the functional consequences of ryanodine interaction with the various postulated binding sites (Chu, Diaz-Munoz, Hawkes, Brush, and Hamilton, 1990), however, very little attention has been paid to the characterization of the ryanodine-modified state.

Obvious analogies with ryanodine modification of SR Ca^{2+} -release channel function are to be found in the actions of a number of naturally occurring toxins on other species of ion channel. The interaction of the alkaloids veratridine and batrachotoxin with voltage-sensitive Na^+ channels results in an increased open probability and a reduced single-channel conductance (Moczydlowski, Garber, and Miller, 1984; Garber and Miller, 1987; Correa, Latorre, and Bezanilla, 1991). The snake venom dendrotoxin peptides and the homologous bovine pancreatic trypsin inhibitor peptide induce subconductance events in maxi Ca^{2+} -activated K^+ channel (Lucchesi and Moczydlowski, 1991). The alterations of single-channel conductance induced by these various toxins most likely occur through allosteric mechanisms in which a conformational rearrangement of the channel protein modulates ion translocation (Garber and Miller, 1987; Correa et al., 1991; Lucchesi and Moczydlowski, 1991; Ravindran, Kwiecinski, Alvarez, Eisenman, and Moczydlowski, 1992).

In the series of experiments reported here, we have set out to identify the mechanisms underlying altered ion handling after ryanodine binding to the SR Ca^{2+} -release channel protein. Towards this end, we have characterized conduction, permeability and block using selected group 1a monovalent, alkaline earth divalent and organic monovalent permeant cations. The parameters obtained in these studies are compared with those found in earlier studies of the unmodified channel and are accommodated into a single-ion occupancy Eyring rate theory model for ion translocation in the channel. As with the modification of Na^+ and Ca^{2+} -activated K^+ channel function by the various toxins described above, we favor an allosteric explanation for the alteration of ion handling induced in the SR Ca^{2+} -release channel by ryanodine.

MATERIALS AND METHODS

Materials

Phosphatidyl-ethanolamine was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and phosphatidylcholine from Sigma Ltd. (Poole, Dorset, UK). [^3H]-ryanodine was obtained from New England Nuclear Ltd., (Boston, MA). Aqueous counting scintillant was purchased from Packard (Groningen, The Netherlands). Ryanodine was obtained from Agrisystems International (Windgap, PA). Other chemicals used were obtained as the best available grade from BDH Ltd (Dagenham, Essex, UK), Aldrich Chemical Company (Gillingham, Dorset, UK) or Sigma Ltd.

Preparation of sheep cardiac heavy sarcoplasmic reticulum (HSR) membrane vesicles. Junctional or heavy sarcoplasmic reticulum membrane vesicles were isolated from the interventricular septum and left ventricular free wall as previously described (Sitsapesan and Williams, 1990). Differential centrifugation of the muscle homogenate provides a mixed membrane fraction, which when fractionated further on a discontinuous sucrose gradient yields a HSR fraction at its 30/40% (wt/vol) interface. The HSR fraction was resuspended in 0.4 M KCl before sedimentation at 36,000 rpm. (100,000 g_{av}) for 1 h in a Sorvall A641 rotor. The resulting pellet was resuspended in a solution containing 0.4 M sucrose, 5 mM *N*'-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES) titrated to pH 7.2 with tris (hydroxymethyl)-methylamine (Tris) and then snap frozen in liquid nitrogen for storage overnight at -80°C .

Solubilization and Separation of the Ryanodine Receptor

The solubilization of the ryanodine receptor by the zwitterionic detergent 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane sulphonate (CHAPS) and subsequent separation of the receptor from other SR membrane proteins was performed as described previously (Lindsay and Williams, 1991). HSR membrane vesicles were solubilized, on ice for 1 h, with 0.5% (wt/vol) CHAPS in the presence of 2.5 mg/ml L- α -phosphatidylcholine (PC) and 1 M NaCl, 0.1 mM ethyleneglycolbis(aminoethyl ether)tetra-acetic acid (EGTA), 0.15 mM CaCl₂, 25 mM piperazine *N,N'*-bis(2-ethanesulphonic acid) (PIPES)-NaOH (pH 7.4), at a protein concentration of 2 mg/ml. After sedimentation of all unsolubilized material by centrifugation, separation of the ryanodine receptor from the other solubilized protein components was achieved by centrifugation on a linear 5–25% (wt/vol) sucrose gradient. Fractions were drawn from the bottom of the tube and the fraction containing the receptor protein was identified by comparison with identical tubes containing membrane components incubated in the presence of [³H]-ryanodine during solubilization. The purified ryanodine receptor was then reconstituted into liposomes by dialysis overnight against a buffer (0.1 M NaCl, 0.1 mM EGTA, 0.15 mM CaCl₂, 25 mM PIPES-NaOH pH 7.4) with five changes of solution, for incorporation into planar phospholipid bilayers.

Planar Lipid Bilayer Methods

Lipid bilayers, formed from suspensions of phosphatidylethanolamine in *n*-decane (35 mg/ml), were painted across a 200- μ m diam hole in a polystyrene copolymer partition which separated two chambers referred to as the *cis* (volume 0.5 ml) and *trans* (vol 1.0 ml) chambers. The *trans* chamber was held at virtual ground while the *cis* chamber could be clamped at various holding potentials relative to ground. Current flow across the bilayer was measured using an operational amplifier as a current-voltage converter as described by Miller (1982). Bilayers were formed in solutions of 200 mM KCl, 20 mM HEPES, titrated with KOH to pH 7.4; resulting in a solution containing 210 mM K⁺. An osmotic gradient was established by the addition of a small quantity (usually 50 to 100 μ l) of 3 M KCl to the *cis* chamber. Proteo-liposomes, containing the purified receptor-channel protein, were added to the *cis* chamber and stirred. To induce fusion of the vesicles with the bilayer a second small aliquot (50–100 μ l) of 3 M KCl was added to the *cis* chamber. After channel incorporation, further fusion was prevented by perfusion of the *cis* chamber with 210 mM K⁺. When other group 1a monovalent cations or organic monovalent cations were to be investigated, bilayers were formed as described above but with the appropriate cation substituted for K⁺. When conduction was investigated in symmetrical divalent solutions, channels were incorporated in K⁺ solutions and both *cis* and *trans* chambers perfused with solutions containing the desired divalent. The use of 210 mM concentrations of the various permeant cations ensured that conduction parameters were obtained under essentially saturating ionic conditions (Lindsay, Manning, and Williams, 1991; Tinker and Williams, 1992, 1993a; Tinker, Lindsay, and Williams, 1992c).

The receptor channel incorporates in a fixed orientation in the bilayer; the *cis* chamber corresponds to the cytosolic face of the channel and the *trans* to the luminal (Lindsay and Williams, 1991; Tinker, Lindsay, and Williams, 1992a). In the subsequent discussion, this naming convention will be adopted and current flowing from the cytoplasm to the interior of the SR referred to as positive to ground (Bertl et al., 1992). Channels were activated by contaminant Ca²⁺ (~10 μ M) in the solution bathing the cytosolic face of the channel. Experiments were carried out at room temperature (21 \pm 2°C).

Ryanodine modification was routinely achieved by the addition of 100 nM ryanodine to the *cis* solution. Occasionally higher concentrations (1–5 μ M) were required to obtain modifications

within reasonable time scales. After modification, unbound ryanodine was removed by perfusion.

Single-Channel Data Acquisition and Display

Single-channel current fluctuations were displayed on an oscilloscope and stored on video or DAT tape. For analysis, data were replayed, filtered using an 8 pole Bessel filter and digitized using an AT based computer system (Satori, Intracel, Cambridge, UK). Depending on single-channel current amplitude, data were low-pass filtered at between 0.2–2 kHz and digitised at 1–10 kHz. Single-channel current amplitudes were determined from digitized data. The representative traces shown in the figures were obtained from digitized data acquired from Satori V3.2 transferred as an HPGL graphics file to a graphics software package (CorelDraw, Corel Systems Corporation, Ottawa, Canada) for annotation and printing.

Determination of Relative Permeability

The permeabilities of the cations examined in this study were expressed as permeability ratios relative to K^+ . Permeability ratios for the group 1a cations were calculated as described previously for the channel in the absence of ryanodine using the Goldman-Hodgkin-Katz equation following the determination of reversal potentials (corrected for junction potentials) from I - V relationships monitored under biionic conditions with 210 mM X^+ at the cytosolic face of the bilayer and 210 mM K^+ at the luminal face of the bilayer (Lindsay et al., 1991).

Relative permeability of the organic monovalent cations was also calculated using the Goldman-Hodgkin-Katz equation following the determination of reversal potential under biionic conditions with 210 mM X^+ at the cytosolic face of the bilayer and 210 mM K^+ at the luminal face of the bilayer. Given the low single-channel conductance with some of the organic cations, we determined reversal potentials using an approach developed earlier for the measurement of this parameter in the unmodified channel (Tinker and Williams, 1993a). Using bilayers containing several channels, the reversal potential was taken as the point at which no excess noise or clear channel openings were detectable at high gain and with low frequency filtering. Either side of the zero current potential, discernable noise was generally apparent before clear channel openings became distinguishable at holding potentials further from the reversal potential. Again reversal potentials were corrected for junction potentials.

The permeability of Ba^{2+} relative to K^+ was determined using the Fatt and Ginsborg modification of the Goldman-Hodgkin-Katz equation (Tinker and Williams, 1992). Single-channel current amplitudes were monitored with 210 mM K^+ at both sides of the channel and 20 mM Ba^{2+} in the solution bathing the cytosolic face of the channel. Reversal potential were determined from I - V relationships monitored at positive holding potentials.

Calculation of Ionic Activity

Where appropriate, ionic activities were calculated as described in previous studies (Tinker et al., 1992c; Tinker and Williams, 1993a).

Modeling Conduction

Ion translocation was investigated using a single-occupancy rate theory model previously developed to describe ion handling in the cardiac SR Ca^{2+} -release channel in the absence of ryanodine (Tinker et al., 1992c; Tinker, Lindsay, and Williams, 1993).

RESULTS

With K^+ as charge carrier, the addition of 100 nM ryanodine to the solution bathing the cytosolic face of the sheep cardiac SR Ca^{2+} -release channel results, after a

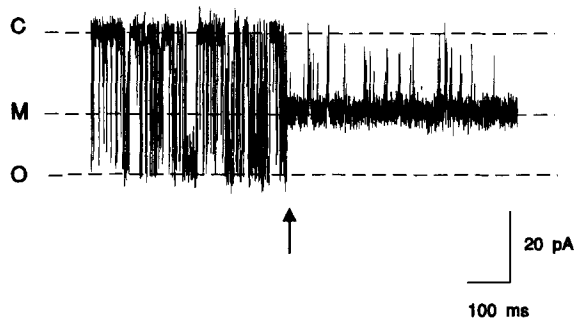


FIGURE 1. Modification of single-channel gating and conductance by ryanodine (*arrow*). Current fluctuations were monitored in symmetrical 210 mM K^+ at a holding potential of 60 mV in the presence of 100 nM ryanodine in the solution bathing the cytosolic face of the channel. (C) Closed level; (M) modified level; (O) open level.

variable period of time, in the modification of both gating and conductance. After the interaction of ryanodine, the channel enters a reduced conductance state of very high open probability (P_o) (Fig. 1). With ryanodine bound, the channel will close both to the true closed level and to intermediate levels, however the probability of closing and the occurrence of incomplete closing events varies with holding potential. At negative holding potentials it is rare to see transitions to the fully closed level, although the channel will enter lower conductance states (Fig. 2). At positive holding potentials we observe transitions into the fully closed state with dwell times in excess of 20 ms, together with many much briefer, often incompletely resolved, closing events. The probability of the channel entering the fully closed state increases as the holding potential is made more positive (data not shown). At high positive holding potentials it is not uncommon for the channel to close for periods of 30 s or more. The variable occurrence of these very long events makes quantification of this apparent voltage dependence of the ryanodine-modified channel extremely difficult and it has not been undertaken in the present study.

The virtual absence of true closing events at negative holding potentials makes determination of single-channel current amplitudes at these voltages very difficult. For this reason we have monitored single-channel current-voltage relationships of the ryanodine-modified channel at positive holding potentials. Fig. 3 shows current fluctuations from a bilayer containing more than one channel before and after the modification of one of the channels by ryanodine. The lower panel of the figure shows current-voltage relationships for these channels. Under these conditions slope

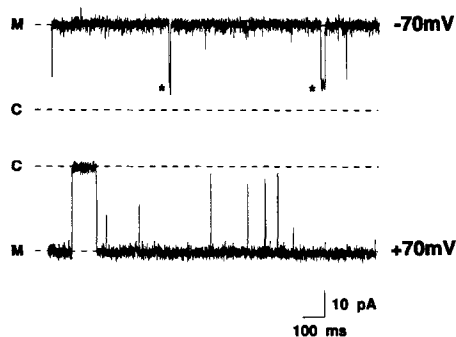


FIGURE 2. Single-channel current fluctuations of a ryanodine-modified channel in symmetrical 210 mM K^+ at holding potentials of ± 70 mV. At positive holding potentials it is common to observe fluctuations to the true closed level. At negative holding potentials true closings are rare and fluctuations occur more often to reduced but not closed current levels. Two such events are marked by the asterisks. (C) Closed level; (M) modified level.

conductance determined by linear regression was 724 ± 7.0 pS ($n = 9$; \pm SEM) for the unmodified channel and 461 ± 10.1 pS ($n = 8$; \pm SEM) for the modified channel.

The aim of this study is to characterize ion handling in the ryanodine-modified cardiac SR Ca^{2+} -release channel. To achieve this we have monitored single-channel conduction, affinity and relative permeability with three classes of permeant ion.

Fractional-Conductance in the Ryanodine-modified Channel

Group 1a monovalent cations. A wide variety of inorganic monovalent cations are permeant in the unmodified cardiac SR Ca^{2+} -release channel (Lindsay et al., 1991). Are these ions still capable of carrying charge following modification of the channel by ryanodine? Fig. 4 a shows single-channel current fluctuations of ryanodine-modified channels at a holding potential of +70 mV in 210 mM symmetrical

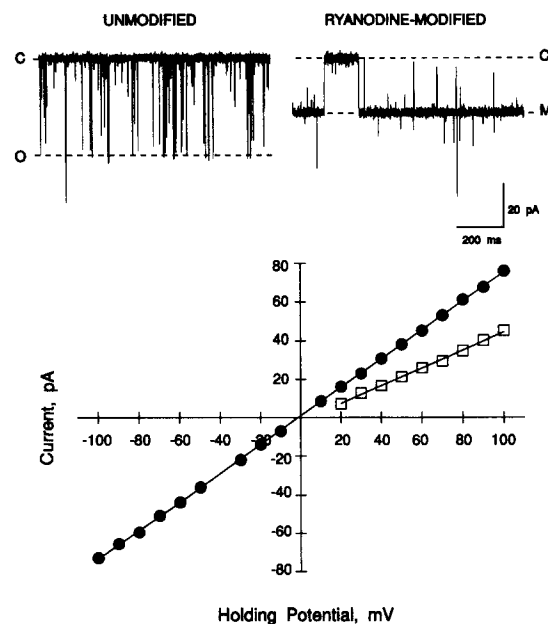


FIGURE 3. The top shows current fluctuations recorded in symmetrical 210 mM K^+ at a holding potential of 60 mV from a bilayer containing more than one channel before and after the modification of one of the channels by ryanodine. (C) Closed level; (M) modified level; (O) open level. The lower panel shows the single-channel current-voltage relationships for the unmodified (\bullet) and ryanodine-modified (\square) channel. Mean conductance values for unmodified and modified channels are quoted in the text.

solutions of Li^+ , Cs^+ , Na^+ , and K^+ . While all of these cations are clearly charge carriers, single-channel current amplitude does vary. Fig. 4 b shows representative single-channel current-voltage relationships for these ions. Conductance values derived from linear regression of such relationships are given in Table I together with the fractional conductance (FC) which is the conductance of the ions in the ryanodine-modified channel as a proportion of their conductance in the unmodified channel (Lindsay et al., 1991). The fractional conductance within the group 1a monovalents is essentially the same.

Alkaline Earth Divalent Cations

Investigations of the conductance of divalent cations in the SR Ca^{2+} -release channel are complicated by the influence of some of these ions on the gating mechanisms of

this channel. High millimolar concentrations of both Ca^{2+} and Mg^{2+} will abolish channel opening when present at the cytosolic face of the channel. However it is possible to study the channel in symmetrical 210 mM solutions of Ba^{2+} and Sr^{2+} . As is the case with the group 1a monovalents as charge carriers, under these conditions, the addition of 100 nM ryanodine to the solution bathing the cytosolic face of the channel results in the occurrence of a characteristic reduced conductance state. With high concentrations of divalent cations, the likelihood of channel closing from the ryanodine-modified conductance state, at either positive or negative holding potentials, is so low as to make evaluation of current-voltage relationships impractical. For this reason we have determined the fractional conductance of the ryanodine-modified channel in divalent cations by monitoring the change in single-channel conductance

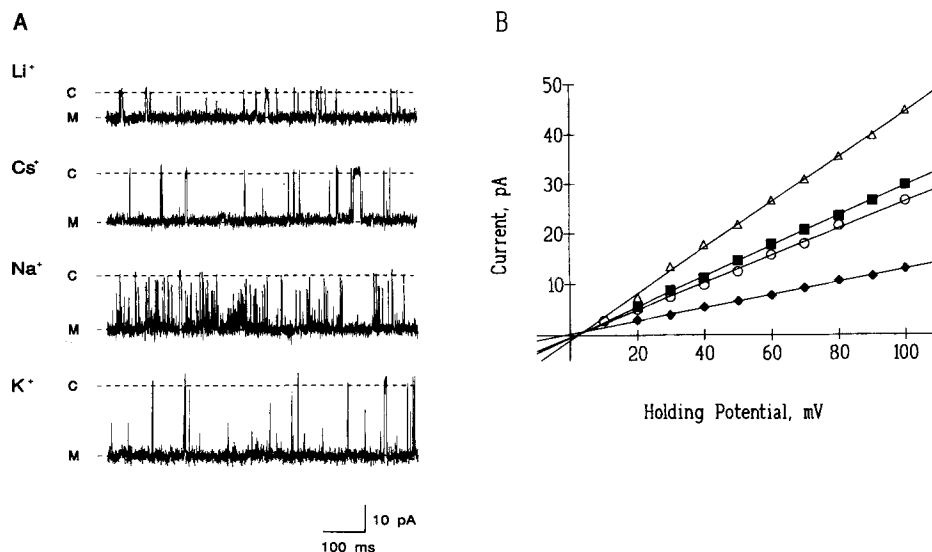


FIGURE 4. (A) Current fluctuations of ryanodine-modified single channels monitored at a holding potential of 70 mV in symmetrical 210 mM solutions of Li^+ , Cs^+ , Na^+ , and K^+ . (C) closed level; (M) modified level. (B) Representative ryanodine-modified single-channel current-voltage relationships monitored in symmetrical 210 mM Li^+ (◆), Cs^+ (○), Na^+ (■) and K^+ (△). Mean conductance values for modified channels are quoted in Table I.

on modification at 40 mV. An example of a modification with Sr^{2+} as the permeant ion is shown in Fig. 5. After the interaction of ryanodine with the channel, chord conductance with Sr^{2+} changes from a mean value of 154.2 to 43.8 pS, while with Ba^{2+} as the permeant cation, chord conductance changes from a mean value of 206.1 to 44.2 pS. Calculated values of fractional conductance for Sr^{2+} and Ba^{2+} are given in Table II.

Organic Monovalent Cations

Recent studies have established that the sheep cardiac SR Ca^{2+} -release channel is permeable to a wide range of organic monovalent cations (Tinker and Williams,

TABLE I
Conduction Parameters of Group 1a Monovalent Cations in the Ryanodine-modified
Sheep Cardiac SR Ca^{2+} -Release Channel

Cation	G , pS \pm SEM	FC	E_{rev} , mV \pm SEM	pX^+/pK^+
	n		n	
K^+	461.0 ± 10.1 (8)	0.64	—	—
Na^+	299.6 ± 2.2 (5)	0.66	-2.1 ± 0.3 (5)	1.09
Cs^+	281.0 ± 5.3 (4)	0.61	10.4 ± 0.1 (4)	0.66
Li^+	128.0 ± 1.4 (4)	0.60	3.7 ± 1.1 (5)	0.86

G is the single-channel conductance in symmetrical 210 mM chloride salt of the appropriate cation. FC is the fractional conductance monitored as described in the text.

1993a). We have monitored the influence of ryanodine modification on the conduction of a number of these cations. As with the inorganic divalent cations we have determined fractional conductance by monitoring modification, in this case at 80 mV. Examples of modifications with ammonia, propanolamine, diethanolamine and diethylamine are given in Fig. 5. Chord conductance for these cations before and after the interaction of ryanodine are as follows; ammonia 562.2 and 373.6 pS, ethylamine 116.5 and 85.6 pS, propanolamine 58.1 and 39.2 pS, diethanolamine 30.5 and 27.8 pS and diethylamine 19.6 and 23.7 pS. Calculated values of fractional conductance for these cations are given in Table III.

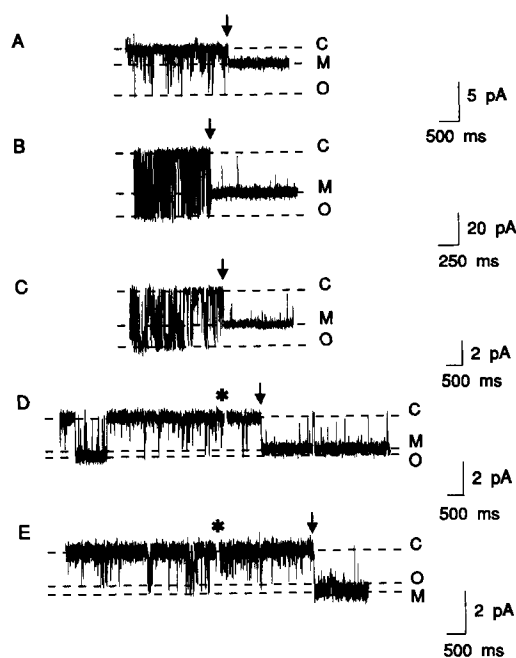


FIGURE 5. Modification of single-channel gating and conduction by ryanodine (arrow) in 210 mM symmetrical solutions of representative alkaline earth divalent and organic monovalent permeant cations. (A) Strontium at a holding potential of 40 mV; (B) ammonia at a holding potential of 60 mV; (C) propanolamine at a holding potential of 80 mV; (D) diethanolamine at a holding potential of 80 mV and (E) diethylamine at a holding potential of 80 mV. D and E contain two sections of trace; in both cases the portion of trace to the left of the break (asterisk) is recorded from the channel before modification and is included to emphasise the current amplitude before ryanodine-modification. In all cases, C indicates the closed level; M, the modified level and O, the open level. Mean fractional conductance values are quoted in Tables II and III.

TABLE II
Conduction Parameters of Alkaline Earth Divalent Cations in the Ryanodine-modified
Sheep Cardiac SR Ca^{2+} -Release Channel

Cation	$FC \pm \text{SEM}$	$E_{\text{rev}}, \text{mV} \pm \text{SEM}$	$\text{pX}^{++}/\text{pK}^{+}$
	<i>n</i>	<i>n</i>	
Ba^{2+}	0.22 ± 0.01 (3)	-13.7 ± 2.4 (7)	3.48
Sr^{2+}	0.28 ± 0.01 (4)	—	—

The information obtained with the various representatives of the three classes of permeant cation demonstrate that while ryanodine modification always results in a channel with an extremely high P_o , the fractional conductance after modification is dependent upon the charge carrier. The tested group 1a monovalent cations display fractional conductances at ~ 0.65 , while fractional conductance with divalent cations as the permeant ion is significantly lower. Interestingly, the fractional conductance with organic cations shows marked variation within the class (Table III) and demonstrates that, under the appropriate ionic conditions, ryanodine modification can result in an increase, rather than a decrease in single-channel conductance.

Affinity of the Ryanodine-modified Channel for Permeant Cations

We have investigated the affinity of the ryanodine-modified channel for a typical group 1a monovalent cation, K^{+} , by studying single-channel conductance, determined from current-voltage relationships between 20 and 100 mV, in a series of symmetrical solutions with concentrations ranging from 10 to 210 mM. This relationship is shown in Fig. 6. Single-channel conductance saturates with increasing K^{+} activity and the relationship can be described in terms of Michaelis-Menten kinetics so that

$$G = G_{\text{max}} / (1 + K_D / [\text{K}^{+}]) \quad (1)$$

where G is the single-channel conductance at the appropriate activity, G_{max} is the maximum conductance and K_D is the activity at which half-maximal conductance

TABLE III
Conduction Parameters of Organic Monovalent Cations in the Ryanodine-modified
Sheep Cardiac SR Ca^{2+} -Release Channel

Cation	$FC \pm \text{SEM}$	$E_{\text{rev}}, \text{mV} \pm \text{SEM}$	$\text{pX}^{+}/\text{pK}^{+}$
	<i>n</i>	<i>n</i>	
Ammonia	0.66 ± 0.01 (5)	-6.5 ± 0.47 (4)	1.29
Ethylamine	0.76 ± 0.01 (5)	16.5 ± 1.35 (4)	0.52
Propanolamine	0.65 ± 0.02 (6)	23.8 ± 0.74 (4)	0.39
Diethanolamine	0.92 ± 0.01 (5)	30.8 ± 1.14 (4)	0.30
Diethylamine	1.21 ± 0.05 (3)	27.7 ± 1.00 (6)	0.33

occurs. The solid curve plotted in this figure was drawn according to Eq. 1 with values of $K_D = 11.6 \pm 1.3$ mM and $G_{\max} = 492 \pm 13.4$ pS obtained by nonlinear regression. Comparison of these parameters with those obtained by us in earlier equivalent studies of the unmodified channel (Lindsay et al., 1991; Tinker et al., 1992c) ($K_D = 20.4 \pm 1.4$ mM, $G_{\max} = 901 \pm 12.7$ pS, Fig. 6, *dashed curve*) indicate that modification of the channel by ryanodine produces a reduction in maximum K^+ conductance and an increase in the affinity of the channel for this cation.

We have employed an alternative approach to determine the relative affinity of both the unmodified and the ryanodine-modified cardiac SR Ca^{2+} -release channels for the permeant organic monovalent cation diethylamine. If two permeant ions compete in a simple fashion at a binding site in the conduction pathway of the channel, the symmetrical addition of increasing concentrations of a cation with low conductance, such as diethylamine, to a channel in symmetrical 210 mM K^+ at a given holding potential, will result in a decrease of single-channel current which will be described by the following relationship

$$\left(1 - \frac{I}{I_c}\right) = r_{\text{limit}} \cdot \frac{[X^+]}{[X^+] + K_D} \quad (2)$$

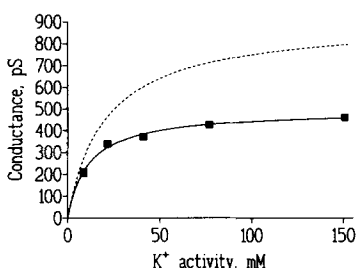


FIGURE 6. Variation in single, ryanodine-modified, channel conductance with K^+ activity. Single-channel slope conductance was monitored as described in Fig. 3 in symmetrical solutions of K^+ at concentrations ranging from 10 to 210 mM. K^+ activity was calculated as described in the Methods section. The solid line is the best fit rectangular hyperbola drawn with the parameters obtained by nonlinear regression as indicated

in the text. The SEM is either included within the symbol or shown by error bars. The dashed line is the best fit rectangular hyperbola for data obtained from channels prior to modification by ryanodine (data from Lindsay et al., 1991) drawn with the parameters given in the text.

where $[X^+]$ is the concentration of diethylamine, I_c is the current in 210 mM K^+ in the absence of diethylamine at the holding potential of 60 mV, I is the current in the presence of $[X^+]$ diethylamine, r_{limit} is the ratio of the current at 210 mM diethylamine, which approaches the saturating current of this cation, relative to K^+ and K_D is a measure of the apparent affinity of the channel for diethylamine in the presence of K^+ . Fig. 7 shows the relationship of $(1 - I/I_c)$ to diethylamine concentration for both unmodified and ryanodine-modified cardiac SR Ca^{2+} -release channels. The best fit parameters generated by nonlinear regression for block of the unmodified channel are $K_D = 12.77 \pm 0.17$ mM and $r_{\text{limit}} = 0.93 \pm 0.004$. The values obtained for the ryanodine-modified channel are $K_D = 32.28 \pm 1.74$ mM and $r_{\text{limit}} = 0.97 \pm 0.022$. Ryanodine modification results in a marked reduction in the relative affinity of the conduction pathway of the channel for diethylamine.

The Relative Permeability of Cations in the Ryanodine-modified Cardiac SR Ca^{2+} -Release Channel

Group 1a monovalent cations. The permeability of these ions relative to K^+ was determined by monitoring reversal potentials under biionic conditions with 210 mM K^+ at the luminal and 210 mM test cation at the cytosolic face of the channel (see Methods). Reversal potentials and calculated permeabilities relative to K^+ are quoted in Table I. Comparison of the data obtained with the group 1a monovalent cations with that obtained for these ions in unmodified cardiac SR Ca^{2+} -release channels ($\text{Na}^+/\text{K}^+ = 1.15$, $\text{Li}^+/\text{K}^+ = 0.99$, $\text{Cs}^+/\text{K}^+ = 0.61$) (Lindsay et al., 1991; Tinker et al., 1992c) indicates that no marked alteration in relative permeability occurs after ryanodine modification.

Relative Permeability of a Representative Divalent Cation, Ba^{2+}

Initial attempts were made to monitor E_{rev} under biionic conditions with 210 mM Ba^{2+} at the cytosolic and 210 mM K^+ at the luminal face of the channel. As was the case with attempts to monitor single-channel current-voltage relationships for

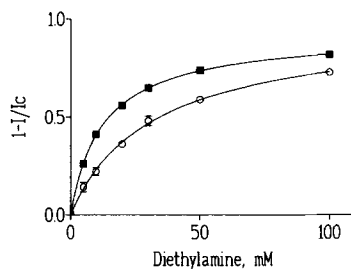


FIGURE 7. The reduction of relative single-channel conductance ($1 - I/I_c$) by diethylamine in symmetrical 210 mM K^+ at a holding potential of 60 mV with unmodified (\blacksquare) and ryanodine-modified (\circ) channels. The solid lines are the best fit rectangular hyperbolas drawn with the parameters obtained by nonlinear regression as indicated in the text. The SEM is either included within the symbol or shown by error bars.

the ryanodine-modified channel in symmetrical high divalent solutions, in the presence of 210 mM cytosolic Ba^{2+} closings from the ryanodine-induced conductance state occurred so rarely as to make accurate measurements of E_{rev} impossible. As a consequence we adopted an alternative approach for the determination of the relative permeability of Ba^{2+} in the ryanodine-modified channel by monitoring E_{rev} with 210 mM K^+ at both sides of the channel and 20 mM Ba^{2+} in the solution bathing the cytosolic face of the channel. The data obtained and the calculated relative permeability of Ba^{2+} are quoted in Table II. The relative permeability of Ba^{2+} obtained in this study (3.48) is considerably lower than the value determined for this parameter with the channel in the absence of ryanodine (5.80) (Tinker and Williams, 1992).

Relative Permeability of Organic Monovalent Cations

As with the group 1a monovalent cations, the permeability of these ions relative to K^+ was determined by monitoring reversal potentials under biionic conditions with 210 mM K^+ at the luminal and 210 mM test cation at the cytosolic face of the channel.

Reversal potentials and calculated permeabilities relative to K^+ are quoted in Table III. These parameters should be compared with the following values obtained previously in the unmodified channel (Tinker and Williams, 1993a); ammonia $E_{rev} = -8.8 \pm 0.2$ mV and $pX^+/pK^+ = 1.42$, ethylamine $E_{rev} = 17.0 \pm 0.6$ mV and $pX^+/pK^+ = 0.51$, propanolamine $E_{rev} = 26.0 \pm 0.2$ mV and $pX^+/pK^+ = 0.35$, diethanolamine $E_{rev} = 40.3 \pm 0.8$ mV and $pX^+/pK^+ = 0.20$ and diethylamine $E_{rev} = 36.3 \pm 0.5$ mV and $pX^+/pK^+ = 0.24$. These data indicate that only very minor variations in relative permeability occur on ryanodine modification with the smaller organic cations ammonia and ethylamine. Application of the t test indicates no significant difference between the relative permeability of ethylamine in the unmodified and ryanodine-modified channel. As the bulk of the organic cation is increased the relative permeability of the ions increases compared to the situation found in the channel before modification by ryanodine; diethanolamine and diethylamine are significantly more permeant in the modified channel (e.g., for diethylamine, $P < 0.001$).

TABLE IV
*The Voltage- and Concentration-independent Energy Profiles (in Multiples of RT)
Used to Model Cation Conduction in the Sheep Cardiac SR Ca^{2+} -Release Channel
before and after Modification by Ryanodine*

Ion	Peak 1	Peak 2	Peak 3	Peak 4	Well 1	C. Well	Well 3
K^+	5.5	5.5	5.5	5.5	-2.35	-3.25	-2.35
K_2	5.5	5.5	5.5	5.5	-2.35	-4.2	-2.35
Ba^{2+}	3.25	3.25	3.25	3.25	-2.35	-9.15	-2.35
Ba	3.9	3.9	3.9	3.9	-2.35	-9.75	-2.35
DE^+	6.9	6.9	6.9	7.1	-5.5	-3.25	-5.25
DE	6.75	6.75	6.75	6.75	-5.5	-3.25	-5.25

DE represent diethylamine. The subscript m identifies the energy profile for the cation after modification of the channel by ryanodine. Various parameters of conduction generated from modeling using these energy profiles are quoted in Table V.

Modeling Ion Conduction in the Ryanodine-modified Cardiac SR Ca^{2+} -Release Channel

In previous investigations of ion conduction, selectivity and block in the unmodified cardiac SR Ca^{2+} -release channel we have interpreted our findings in terms of Eyring rate theory, using a single-occupancy model in which it is envisaged that an ion, travelling through the conduction pathway, crosses four major energy peaks and interacts with three binding sites (Tinker et al., 1992c; Tinker et al., 1993; Tinker and Williams, 1993a). Can ion conduction in the ryanodine-modified channel be described using this basic model? In an effort to answer this question we have attempted to model ion handling in the ryanodine-modified channel by altering the energy profiles used in our earlier studies for a representative group 1a monovalent cation, K^+ and a representative divalent cation, Ba^{2+} . In addition, we have extended the study by deriving energy profiles for diethylamine in both the unmodified and

TABLE V
Comparison of Observed Conduction Parameters with Those Predicted from
Calculations Using the Energy Profiles for the Various Cations Shown in Table IV

Ion	E_{rev} , mV	G_{210} , pS	FC	K_D , mM	G_{max}/V_{max}
<i>observed/predicted</i>					
K_m^+	—/—	461/443	0.64/0.59	11.6/12.7	492/480
Ba_m^{2+}	-13.7/-12.6	NM	0.22/0.29	NM/0.091	NM
DE^+	36.3/34.0	19.4/17.8	—/—	12.8/15.6	0.93/0.95
DE_m^+	27.7/29.6	NM	1.22/1.25	32.3/28.7	0.97/0.92

DE represent diethylamine. The subscript *m* identifies data obtained for ryanodine-modified channels. NM indicates that the parameter has not been measured or calculated.

ryanodine-modified cardiac SR Ca^{2+} -release channel. The details of the energy profiles obtained are given in Table IV and a comparison of various observed parameters with those predicted using the profiles detailed in Table IV is given in Table V.

The energy profile derived for diethylamine in the unmodified channel is similar, in general form, to those used by us recently for the other permeant organic cations trimethylamine and diethylmethylamine (Tinker and Williams, 1993a). For all three organic cations the energy peaks encountered are somewhat higher than those seen by the inorganic monovalent and divalent cations and the organic cations are assumed to bind with higher affinity at wells 1 and 3.

As is clear from the information presented in Table V, relatively minor modifications of our basic single-occupancy four peak, three well rate theory model for ion conduction in the unmodified channel are sufficient to provide a very adequate fit to the data with representatives of three classes of permeant cation in the ryanodine-modified channel. Further support for this assertion comes from experiments in which we have investigated the block of K^+ conductance by another permeant cation, Ba^{2+} . Fig. 8 shows the influence of increasing concentrations of Ba^{2+} added symmetrically to ryanodine-modified channels with 210 mM K^+ as permeant ion at both the cytosolic and luminal faces of the channel. Conductance was determined at a holding potential of 60 mV and expressed relative to the conductance in the absence of Ba^{2+} . The curve drawn through the points was derived from a computer

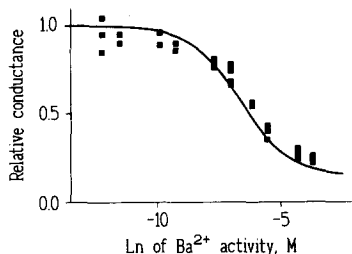


FIGURE 8. The relative conductance of single, ryanodine-modified, channels monitored in symmetrical 210 mM K^+ at a holding potential of 60 mV in the presence of symmetrical Ba^{2+} (0–40 mM). Each point represents an individual experiment. The solid line is a fit generated from a four barrier, three well rate theory model using the energy profiles for K^+ and Ba^{2+} given in Table IV.

simulation of the experiment using the energy profiles for K^+ and Ba^{2+} in the ryanodine-modified channel given in Table IV. Again, under quite complex conditions involving the interaction of two permeant ions with the channel, there is good agreement between the observed data and that predicted from the model.

DISCUSSION

To appreciate how ryanodine might modify channel function we must first consider how cation conduction, selectivity and block are achieved in the cardiac SR Ca^{2+} -release channel in the absence of ryanodine. Our previous studies suggest that the conduction pathway of the unmodified cardiac SR Ca^{2+} -release channel contains: a selectivity filter with a radius of ~ 3.5 Å, located at $\sim 10\%$ into the voltage drop from the luminal face of the channel (Tinker and Williams, 1993a); a hydrophobic binding

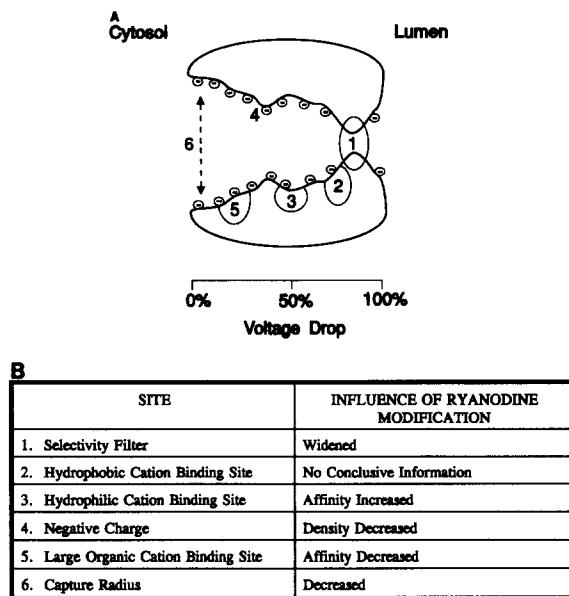


FIGURE 9. A cartoon of a portion of the conduction pathway of the SR Ca^{2+} -release channel corresponding to the region over which transmembrane voltage falls. The various sites indicated on the cartoon have been identified in previous studies from this laboratory (see text for details) and are documented in the table in the bottom of the figure which also contains a summary of the alterations which we believe occur on modification by ryanodine.

site adjacent to the cytosolic side of the selectivity filter (Tinker et al., 1992a); a high field strength binding site for monovalent and divalent cations located $\sim 50\%$ into the voltage drop (Tinker et al., 1992a, 1992c) and a wide vestibule at the cytosolic limit of the voltage drop (Tinker et al., 1992b; Tinker and Williams, 1993b). In addition, it is probable that there is a high density of negative charge throughout the conduction pathway (Tinker et al., 1992c). These features are summarized in Fig. 9 which is a highly stylized cartoon of the conduction pathway of the channel. The structure shown in this figure corresponds to the region of the pore of the channel over which the transmembrane voltage falls. In reality this will almost certainly represent only a small part of the structure seen in negatively stained images of the channel in electronmicrographs (Wagenknecht, Grassucci, Frank, Saito, Inui, and

Fleischer, 1989; Radermacher, Wagenknecht, Grassucci, Frank, Inui, Chadwick, and Fleischer, 1992).

The channel is permeable to both divalent alkaline earth and group 1a monovalent cations. Within their respective groups the divalent and monovalent cations are essentially equally permeant in the channel. However the channel does select for divalent cations over monovalent cations; in general terms $pX^{++}/pY^{+} \approx 6$. While, within a group, there is little difference in the relative permeability of the group 1a monovalents or the relative permeability of the alkaline earth divalents, the single-channel conductance of these cations does vary over a wide range. For both divalent and monovalent permeant cations, single-channel conductance saturates with increasing ionic activity and the relationship can be described in terms of a simple single-site scheme.

These characteristics can be qualitatively and quantitatively reproduced using a simple Eyring rate theory model in which we assume that the conduction pathway of the channel can be occupied by no more than one ion at a time. Discrimination between divalent and monovalent cations results from interaction with the central high affinity binding site and a process that favors the passage of divalent cations between binding sites in the conduction pathway. The different single-channel conductance seen within the alkaline earth divalent cations and within the group 1a monovalent cations is explained by differing affinity for the ions at this central binding site (Tinker et al., 1992c). Transport rates of large organic cations are determined by sieving at the selectivity filter and the degree of interaction with the hydrophobic binding site at the cytosolic side of this filter (Tinker and Williams, 1993a).

Therefore, we can separate the cations which are permeant in the SR Ca^{2+} -release channel into three classes; the alkaline earth divalent cations, the group 1a monovalent cations and the large organic monovalent cations. In broad terms, the mechanisms governing ion-handling of these cations are the same within a class but differ between classes. The interaction of ryanodine with the cardiac SR Ca^{2+} -release channel brings about dramatic alterations to this pattern of ion-handling, the most obvious of which is a change in single-channel conductance. What mechanisms can we propose to explain this observation?

It is possible that the functional Ca^{2+} -release channel consists of a number of separate ion translocation pathways that gate in concert; for example four, with one pathway provided by each monomer of the homotetramer (Wagenknecht et al., 1989; Williams, 1992). An intuitive interpretation of the mechanism leading to the reduced conductance state following ryanodine modification might be that the alkaloid selectively blocks a number of these conduction pathways. However, the variation in fractional conductance with ionic species and the increase in this parameter with diethylamine clearly excludes this possibility.

More subtly, is there any means by which a form of "partial block" of the conduction pathway due to ryanodine binding at the cytosolic vestibule of the pore may explain the derived conduction parameters? Indeed we have postulated that just such a phenomenon may occur with other, reversible, modulators of conduction in

this channel (Tinker et al., 1992*b*; Tinker and Williams, 1993*b*). Partial block arises because of changes in ion capture radius and the electrostatics of ion handling in the vestibule (Dani, 1986; Dani and Fox, 1991). However, it is difficult to envisage how a single alteration of a particular physical factor affecting ion-handling can lead to, for example, an increase in the affinity of the channel for K^+ , decreases in divalent-monovalent relative permeability but an increase in diethylamine- K^+ relative permeability, and variations in fractional conductance with ionic species.

A more likely possibility is that the interaction of ryanodine may produce a change in the conformation of the channel protein, and modified gating and ion-handling of the SR Ca^{2+} -release channel would then represent the functional consequences of this, i.e., an allosteric hypothesis. Similar proposals have been put forward to explain the altered single-channel conductance and permeability observed on modification of voltage-sensitive Na^+ channels by batrachotoxin (Garber and Miller, 1987; Correa et al., 1991; Ravindran et al., 1992). Such a proposal does not necessarily exclude binding of ryanodine within the pore, nor some contribution to the observed effect from partial block. Indeed the Zn^{2+} -induced substate in the batrachotoxin-modified sodium channel is affected by mutations within the pore mouth and yet kinetic evidence points to an allosteric transition (Schild, Favre, and Moczydlowski, 1993). What then might be the consequences of the interaction of ryanodine with the channel protein? To try to tease out possible physical alterations in channel structure we have attempted to explain the data in terms of alterations to our single-ion four barrier three binding site Eyring rate theory model for the unmodified channel (Tinker et al., 1992*c*; Tinker et al., 1993). In single-ion models, the relative permeability depends entirely on differences in peak heights of the energy profile, affinity will be governed by well depth and the observed conductance will result from a combination of these two factors. If we assume that, after the interaction of ryanodine, the channel remains single ion, what alterations are required to explain the modified function of the channel?

Group 1a Monovalent Cations

Following modification by ryanodine, the affinity of the conduction pathway of the channel for K^+ is increased significantly. At the same time the permeabilities of Na^+ , Cs^+ , and Li^+ relative to K^+ are essentially unchanged. Based on this evidence and our existing knowledge of the mechanisms governing ion handling in the channel (Tinker et al., 1992*c*) it would seem probable that the reduction of single-channel conductance observed on ryanodine modification of the channel with group 1a monovalent cations as permeant species can be explained solely by an increase in the affinity of the central binding site, i.e., a lowering of central well depth in the energy profile. Modeling K^+ conduction confirms this hypothesis (Tables IV and V). As the values of fractional conductance obtained with all group 1a monovalent cations were basically the same (0.60–0.66), it seems reasonable to suggest that the conformational rearrangement, resulting from the interaction of ryanodine, has an equivalent effect on the affinity of the central well for all group 1a monovalent cations.

Alkaline Earth Divalent Cations

As a class, divalent cations have a significantly lower fractional conductance than the group 1a monovalent cations. Measurement of the permeability of Ba^{2+} relative to K^{+} indicates that this parameter decreases on modification by ryanodine. Within the context of a single-ion model this can be achieved by raising peak heights in the energy profile, which in itself, would be predicted to reduce single-channel conductance. However, modeling of Ba^{2+} conduction in the ryanodine-modified channel indicates the additional requirement for an increase in affinity of the central binding site to explain the observed fractional conductance. Our earlier investigations of ion conduction in the SR Ca^{2+} -release channel in the absence of ryanodine have shown that divalent cations are bound more tightly to this central site than the group 1a monovalents and it is therefore not surprising that ryanodine-induced conformational rearrangements which lead to increases in affinity for group 1a monovalent cations also result in increased affinity for permeant divalent cations. There is a small but significant difference between the values of fractional conductance seen with Ba^{2+} and Sr^{2+} (Table II), which presumably reflects the slightly different affinities and relative permeabilities of these cations in the unmodified channel (Tinker et al., 1992c).

Organic Monovalent Cations

Unlike the two classes of permeant cations discussed above, the organic monovalent cations examined in this study show a wide variation in fractional conductance (0.65–1.21). Can we explain these findings in terms of modifications of our model for organic monovalent cation handling in the channel in the absence of ryanodine? In earlier studies we established that the relative permeability of the organic cations in the unmodified channel was inversely related to the minimum circular radius of the cation and calculated a minimum pore radius of 3.3–3.5 Å. An examination of the relative permeability data quoted in Table III demonstrates that whilst the relative permeabilities of the two smaller cations investigated (ammonia, radius 1.7 Å and ethylamine, radius 2.2 Å) show only minor variations when compared to their values in the unmodified channel, the larger cations (diethanolamine, 2.8 Å and diethylamine, 2.5 Å) show significant increases in relative permeability on modification. The cation of intermediate size, propanolamine (2.3 Å), shows a small increase in relative permeability consistent with this trend. Taken together, this information might suggest that the interaction of ryanodine with the channel leads to a decrease in the energy barriers experienced by the larger of the organic cations as they pass through the conduction pathway. This is confirmed by the modeling data for diethylamine quoted in Tables IV and V. The observed fractional conductance can be produced in the model by a lowering of all peak heights and in particular a lowering of peak 4, which is equivalent to a modification of the postulated selectivity filter in the unmodified channel; no alteration in well depth is necessary to account for the observed alterations in conduction parameters. Given this, the apparent change in K_D for diethylamine monitored in our experiments (Fig. 7) and predicted by the model (Table V) appears, at first sight, to be anomalous. The K_D values given in the table were determined by monitoring the relative affinity of diethylamine from experi-

ments, or model reconstructions of experiments, in which this cation reduced K^+ current in a simple competitive fashion. The apparent discrepancy between the alteration in apparent affinity on ryanodine modification and the lack of need to change central well depth in the energy profile for this cation results from the increased affinity of the channel for K^+ after the interaction of ryanodine.

Whilst the lowering of the energy barriers for the larger organic cations appears to provide a plausible explanation for the gross differences in fractional conductance observed between small and large organic cations, it does not provide a complete explanation for organic cation handling after ryanodine modification. Why is the fractional conductance for diethylamine greater than that of the larger cation diethanolamine? In our rate theory model, size-dependent sieving was not sufficient, in itself, to explain variations in single-channel conductance with the organic cations in the channel in the absence of ryanodine. Conductance was also influenced by binding of the cations to sites within the conduction pathway (Tinker and Williams, 1993a). Of particular importance are the interaction of the cations with the central (hydrophilic) site and a hydrophobic site (Well 3). The relative importance of these sites will obviously vary with the nature of the cation, hydrophobic cations will be excluded from the central site but bound at Well 3; hydrophilic cations will be more influenced by binding to the central site. Modeling of diethylamine conduction in the ryanodine-modified channel suggests that the binding of the alkaloid to the channel, while it reduces the barriers for translocation, has no influence on binding to either of these sites (Table IV). Although a larger cation, diethanolamine can be considered as considerably less hydrophobic than diethylamine and as such is likely to be influenced by the increase in central site affinity induced by ryanodine binding.

Therefore, the factors regulating organic cation fractional conductance after ryanodine modification of the channel are complex and are dependent upon both the size and the nature of the cation. Ammonia is handled in much the same way as a group 1a monovalent cation; as the minimum circular radius of the cation is increased the passage of the cation through the conduction pathway will be influenced by sieving as well as interactions with the hydrophilic and hydrophobic binding sites described above.

Structural Consequences of Ryanodine Modification

The preceding discussion adds support to the suggestion that the binding of ryanodine to the cardiac SR Ca^{2+} -release channel results in a conformational change in the channel protein with an ensuing alteration of the voltage- and concentration-independent energy profiles experienced by cations as they are translocated across the conduction pathway. Can we propose a structural basis for these observations? The increased rate of transport of the organic cation, diethylamine, is achieved by reducing all four peaks in the energy profile. The largest reduction is made to peak 4 which we have previously postulated as being equivalent to the selectivity filter (Tinker and Williams, 1993a). In physical terms, this could be achieved by a widening of the filter; calculation of minimum pore radius of the channel after ryanodine modification using excluded area theory for the organic cations examined here suggests a 0.2–0.3 Å increase from the 3.3–3.5 Å determined for the channel in the absence of ryanodine (Tinker and Williams, 1993a). As observed, an increase in

minimum pore radius would be expected to increase the relative permeability of the larger organic cations but would have little or no effect on the relative permeability of the group 1a or small organic monovalent cations.

Our findings indicate that the affinity of the central cation binding site of the conduction pathway increases for both alkaline earth divalent and group 1a monovalent cations. We have previously demonstrated that this site possesses the characteristics of a high field strength site and have postulated that such a site might involve oxygens of high field strength carboxyl groups (Tinker et al., 1992c). A structural reorganization resulting in an increase in the field strength of such a site would be consistent with our current observations.

In the unmodified channel, discrimination between divalent and monovalent cations results from differential binding at the central site and a process which favours the passage of divalent cations between binding sites. A physical correlate of the latter system could be provided by a high density of negatively charged residues in the conduction pathway. A high charge density will favour divalent over monovalent cation permeability (Tinker et al., 1992c). Ryanodine interaction with the channel produces a decrease in the relative permeability of Ba^{2+} , with no significant alteration in the relative permeability of the group 1a monovalent cations. A structural alteration of the conduction pathway which produces a lowering of the density of negative charge would be expected to result in this pattern of events. Such a redistribution of charge could occur if the surface area of the lining of the conduction pathway increased and would be consistent with a structural reorganization that resulted in an increase in minimum pore radius.

Additional support for the concept of a structural reorganization after ryanodine binding comes from our earlier investigation of the block of unmodified and ryanodine-modified channels by large impermeant organic cations such as tetrabutyl ammonium (TBA^+) (Tinker and Williams, 1993c). These observations are consistent with an alteration of the structure of the cytosolic vestibule of the channel after the interaction of ryanodine that results in a reduction in the capture radius of the channel for blocker entry.

The various postulated alterations to the conduction pathway of the cardiac SR Ca^{2+} -release channel induced by the high affinity interaction of ryanodine are summarized in Fig. 9.

Conclusion

We believe that the data described in this paper indicate that on binding, ryanodine induces an alteration of the structure of the cardiac SR Ca^{2+} -release channel and we have presented arguments which go some way towards providing a mechanistic interpretation of the ways in which changes in the conformation of the channel protein could result in the observed parameters of cation conduction and discrimination. With the majority of the cations examined in this study, the interaction of ryanodine with the channel produces a reduction in the rate of ion translocation. Surprisingly, although conduction is reduced, the channel in broad terms, becomes less selective. The observed reduction in single-channel conductance results from a more significant increase in the affinity of binding sites within the conduction pathway. While we have not carried out a detailed investigation of the mechanisms

underlying the dramatic alterations in channel gating after ryanodine binding, it seems reasonable to assume that these also result from a reorganisation of channel structure.

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