Probing the Structure of the Conduction Pathway of the Sheep Cardiac Sarcoplasmic Reticulum Calcium-release Channel with Permeant and Impermeant Organic Cations

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ABSTRACT The sarcoplasmic reticulum Ca\(^{2+}\)-release channel plays a central role in cardiac muscle function by providing a ligand-regulated pathway for the release of sequestered Ca\(^{2+}\) to initiate contraction following cell excitation. The efficiency of the channel as a Ca\(^{2+}\)-release pathway will be influenced by both gating and conductance properties of the system. In the past we have investigated conduction and discrimination of inorganic mono- and divalent cations with the aim of describing the mechanisms governing ion handling in the channel (Tinker, A., A. R. G. Lindsay, and A. J. Williams. 1992. Journal of General Physiology. 100:495-517.). In the present study, we have used permeant and impermeant organic cations to provide additional information on structural features of the conduction pathway. The use of permeant organic cations in biological channels to explore structural motifs underlying selectivity has been an important tool for the electrophysiologist. We have examined the conduction properties of a series of monovalent organic cations of varying size in the purified sheep cardiac sarcoplasmic reticulum Ca\(^{2+}\)-release channel. Relative permeability, determined from the reversal potential measured under bi-ionic conditions with 210-mM test cation at the cytoplasmic face of the channel and 210 mM K\(^+\) at the luminal, was related inversely to the minimum circular cation radius. The reversal potential was concentration-independent. The excluded area hypothesis, with and without a term for solute-wall friction, described the data well and gave a lower estimate for minimum pore radius of 3.3-3.5 Å. Blocking studies with the impermeant charged derivative of triethylamine reveal that this narrowing occurs over the first 10-20% of the voltage drop when crossing from the lumen of the SR to the cytoplasm. Single-channel conductances were measured in symmetrical 210 mM salt. Factors other than relative permeability determine conductance as ions with similar relative permeability can have widely varying single-channel conductance. Permeant ions, such as the charged derivatives of trimethylamine and diethylmethylamine, can also inhibit K\(^+\) current. The
reduction in relative conductance with increasing concentrations of these two ions at a holding potential of 60 mV was described by a rectangular hyperbola and revealed higher affinity binding for diethylmethylamine as compared to trimethylamine. It was possible to describe the complex permeation properties of these two ions using a single-ion four barrier, three binding site Eyring rate theory model. In conclusion, these studies reveal that the cardiac Ca\(^{2+}\)-release channel has a selectivity filter of \(~3.5\text{-Å}\) radius located at the luminal face of the protein. Transport rates for organic cations are determined by sieving according to size at the selectivity filter, with specific chemical factors playing only a small role, and a hydrophobic binding site located just after this as cations pass from the lumen of the sarcoplasmic reticulum to the cytosol.

**INTRODUCTION**

The study of excitation-contraction coupling, in both skeletal and cardiac muscle, has been advanced by the identification of the ion channel responsible for the release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR). Initially studies of single-channel properties were made on native heavy SR membrane vesicles reconstituted into planar phospholipid bilayers in ionic conditions in which current deflections could be unambiguously attributed to the Ca\(^{2+}\)-release channel. The use of the specific high affinity ligand \(^{3}[H]\)-ryanodine has led to the development of purification protocols and subsequent electrophysiological study in planar bilayers (Imagawa, Smith, Coronado, and Campbell, 1987; Smith, Imagawa, Ma, Fill, Campbell, and Coronado, 1988; Lai, Erickson, Rousseau, Liu, and Meissner, 1988; Anderson, Lai, Liu, Rousseau, Erickson, and Meissner, 1989). Our laboratory has used such a preparation to study the mechanisms of ion discrimination and block in the sheep cardiac Ca\(^{2+}\)-release channel (Lindsay and Williams, 1991; Lindsay, Manning, and Williams, 1991; Tinker and Williams, 1992; Tinker, Lindsay, and Williams, 1992b,c). The picture of the channel that has emerged from these studies suggests that ion conduction through the SR Ca\(^{2+}\)-release channel is a relatively simple process with the channel well suited to its physiological role. The channel shows high turnover with both mono- and divalent cations as permeant species and maintains sufficient selectivity between relevant physiological cations (Tinker, Lindsay, and Williams, 1993).

In this communication we examine the conduction properties of a series of monovalent organic cations in the sheep cardiac SR Ca\(^{2+}\)-release channel. What is the rationale for performing such work? The permeation of ions other than those of physiological interest is of importance for several reasons. In particular, the study of permeant and impermeant organic cations can give indications of the importance of ion size and chemical interactions in determining selectivity and also importantly lead to estimates of the dimensions of the narrowest region of the pore (Dwyer, Adams, and Hille, 1980; Coronado and Miller, 1982; McCleskey and Almers, 1985). Such basic studies are becoming increasingly important as ion channel structure-function is investigated at the molecular level (Jan and Jan, 1992; Lester, 1992). The recent cloning of cardiac and skeletal sarcoplasmic reticulum Ca\(^{2+}\)-release channels makes such studies even more pertinent (Takeshima et al., 1989; Zorzato, Fujii, Otsu, Green, Lai, Meissner, and MacLennan, 1990; Otsu, Willard, Khanna, Zorzato, Green,
and MacLennan, 1990). A second reason is that ionic substitution to eliminate ionic current carried through particular channels or carriers has been important in elucidating physiological mechanisms underlying excitability and other biological processes. The ability to isolate elements of the SR by such an approach would be of obvious interest. Thirdly on a more biophysical note, the data may shed light on the general relationship between permeation and block.

The results presented in this communication indicate that the cardiac Ca\(^{2+}\)-release channel is permeable to a wide range organic cations and has a relatively wide selectivity filter, with organic cation permeability determined by sieving according to size. Net transport rate is also significantly influenced by cations binding within the conduction pathway. Comparison of these properties and those determined from our previous studies with similar work on other cation selective channels indicates that the “finger-print” of ion permeation shown by the cardiac SR Ca\(^{2+}\)-release channel, though bearing some resemblance to the family of neurotransmitter gated channels, is essentially unique.

**METHODS**

*Materials*

Phosphatidylethanolamine was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and phosphatidylcholine from Sigma Ltd. (Poole, Dorset, UK). \(^{3}H\)-ryanodine was obtained from New England Nuclear (Boston, MA). Aqueous counting scintillant was purchased from Packard (Groningen, The Netherlands). The organic cations studied were obtained from Aldrich Chemical Company (Gillingham, Dorset, UK) or Janssen Chimica (Beerse, Belgium). The compounds were obtained as the free amines and the requisite amount added to deionized water buffered with 20 mM N\(^{-}\)2-hydroxyethylpiperazine-N\(^{-}\)2-ethanesulphonic acid (HEPES). The free amine was subsequently titrated to pH 7.4 with concentrated hydrochloric acid giving a chloride salt of the amine. All the compounds studied were highly basic and at pH 7.4 were present as the charged cation. When used in blocking experiments, the relevant ions were dissolved in the standard experimental 210 mM K\(^{+}\) solution to make concentrated stock solutions from which small aliquots could be added to the solutions in the cis and trans chambers. 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. (Poole, Dorset, UK). The cations are referred to by the name of the uncharged derivative and a list of those studied is given in Table I.

*Preparation of the Purified Sheep Cardiac SR Ca\(^{2+}\)-Release Channel*

The sheep cardiac Ca\(^{2+}\)-release channel was purified as previously described (Lindsay and Williams, 1991).

*Planar Lipid Bilayer Methods*

Lipid bilayers, formed from dispersions of phosphatidylethanolamine in \(\pi\)-decane (35 mg/ml), were painted across a 200-\(\mu\)m diam hole in a polystyrene copolymer partition which separated two chambers referred to as the cis (vol 0.5 ml) and trans (vol 1.5 ml) chambers. The trans chamber was held at virtual ground whilst 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 (1982b). For the
measurement of moderate or large current deflections (equivalent to a conductance of approximately 60 pS or more under symmetrical conditions) a head amplifier with a fast rise time (≈ 50–200 μs) was used. Rather than heavily filter data for small current deflections (equivalent to a conductance of < 60 pS under symmetrical conditions) a head amplifier with a much slower rise time was used (≈ 5 ms). Under such conditions, given the nature of the gating of the sheep cardiac Ca^{2+}-release channel, it was possible to resolve single-channel fluctuations with conductances as low as 10 pS.

Bilayers were formed under three conditions. For the measurement of reversal potentials and for blocking experiments, bilayers were formed in solutions of 210 mM K^+ (200 mM KCl, 20 mM HEPES, titrated with KOH to pH 7.4). For the measurement of conductance in a symmetrical solution of the relevant organic cation bilayers were formed either as above (with subsequent perfusion of both chambers) or in the cation under study. Subsequent analysis of single-channel conductance for both methods of incorporation gave identical results. For examination of the concentration dependence of the reversal potential with varying K^+ concentration, bilayers were formed in the K^+ concentration of interest.

An osmotic gradient was established by the addition of a small quantity (usually 50–100 μl) of 3 M KCl to the cis chamber. Proteo-liposomes were added to the cis chamber and stirred. To induce fusion of the vesicles with the bilayer a second small aliquot (50–100 μl) of 3 M KCl was added to the cis chamber. After channel incorporation, further fusion was prevented by perfusion of the cis chamber with the cation of interest to obtain the desired ionic conditions. Solutions contained 10 μM free Ca^{2+} as contaminant which was generally sufficient for channel activation. Very occasionally, this was raised to 50–100 μM to increase single-channel open probability. Studies were generally carried out on bilayers containing two to three channels and sometimes more. Experiments were carried out at room temperature (21 ± 2°C).

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 et al., 1992c). 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).

**Single-Channel Data Acquisition and Analysis**

Single-channel current fluctuations were displayed on an oscilloscope and stored on videotape. For analysis, data were replayed, filtered using an 8 pole Bessel filter and digitized using an AT based computer system (Satori, Intracei, Cambridge, UK). Data were filtered at rates between 200 and 1,000 Hz depending on the single-channel conductance being measured. Digitization rates were set to at least four times the filtering rate. Single-channel current amplitudes were determined from digitized data measured by on screen cursors. 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.

Nonlinear and linear regression fits to the data of the equations detailed later in the text were calculated using a commercially available software package (Graphpad Inplot v 4.03, GraphPad Software, San Diego, CA). The nonlinear regression program generates standard errors of the mean (SEM). However these figures should be treated cautiously as only approximate measures of data spread.

**Calculation of Molecular Dimensions of the Studied Organic Cations**

The molecular dimensions of the organic cations studied in this paper were determined using a commercially available molecular modelling software package (HyperChem Release 2, Autodesk Inc, Sausalito, CA). The relevant structures were minimised solvated in water using the
MM+ force field. The minimum circular area was determined for each cation by the use of a mouse controlled cursor. The radii obtained are given in Table I. The figures are broadly similar to those determined by more traditional methods for example in Cohen et al. (1992). In general there is a tendency for the radii determined here to be slightly smaller especially for the long chain derivatives (see below). This reflects the method of determination; our method gives the minimum circular radius through which a cation with an optimum geometry can pass whilst most authors have measured a mean radius determined from total ion volume. Hardcopy could be obtained through a print routine and such plots are used in Fig. 8 b.

Calculation of Permeability Ratios

Reversal potentials \( (E_{rev}) \) were determined under bi-ionic conditions. Under such conditions, often with several channels incorporated in the bilayer, the reversal potential was the point at which no excess noise or clear channel openings were visible with high gain and low frequency filtering. Either side of the zero current potential, excess noise was generally detectable before clear channel openings became distinguishable at larger potentials relative to the reversal potential. Given this approach, it was generally possible to measure the reversal potential to within ±2 mV. All reversal potentials were corrected for junction potentials. The permeability ratios for the respective monovalent organic cations relative to K⁺ were calculated from the Goldman-Hodgkin-Katz equation (Hodgkin and Katz, 1949). If X⁺ is a monovalent organic cation present at the cytosolic face of the channel and K⁺ is present at the luminal face then the permeability ratio \( (P_{X^+/P_{K^+}}) \) is given by

\[
\frac{P_{X^+}}{P_{K^+}} = \frac{[K^+]}{[X^+]} e^{-E_{rev}F/RT}
\]

where \( RT/F \) has its conventional meaning and a value of 25.2 mV at 20°C. \([K^+]\) and \([X^+]\) refer to the concentration of the respective ions.

Under some circumstances activities rather than concentrations were required (see text) and these were obtained from either Hamer and Wu (1972) as quoted in Lobo (1989) or from Kieland (1937) as quoted in Kortum and Bockris (1951).

Modeling Ionic Conduction

Ionic conduction was modeled using an approach outlined in Tinker et al. (1992a). In such modeling work ion activities rather concentrations are used. A PC-executable version of the program is available from the authors. Please send a preformatted disc (either 5.25 or 3.5 inches).

RESULTS

Work from our laboratory has previously investigated the conduction and permeability properties of the group Ia monovalent (Lindsay et al., 1991) and the alkaline earth divalent cations (Tinker and Williams, 1992) in the sheep cardiac SR Ca²⁺-release channel. We now extend our study of ionic conduction in this channel to include a survey of selected permeant and impermeant organic cations.

Organic Cation Single-Channel Conductance

A wide range of organic cations are able to carry current in the Ca²⁺-release channel. Fig. 1 shows representative single-channel traces with selected organic cations and Fig. 2 a sample current-voltage relationships in symmetrical 210 mM cation. Table I shows the conductances in symmetrical 210 mM salt of all the cations studied.
Figure 1. Representative single-channel current fluctuations at a holding potential of −80 mV in symmetrical 210 mM salt. Cations are as indicated in the figure. (A) Recorded using a head amplifier with a rise time of 50 μs, filtered at 1 kHz and digitized at 4 kHz. (B) Recorded using a head amplifier with a rise time of 5 ms, filtered at 200 Hz and digitized at 2 kHz. Note the different time scales and gains in A and B.
together with the relevant statistics. Triethylamine, triethanolamine, l-lysine, diethylethanolamine and ethyldiethanolamine had conductances of < 10 pS. Conductances of the ions examined range from 594 and 389 pS for ammonia and hydrazine respectively to 10.4 pS for diethylmethylamine.

The current-voltage relationships are approximately symmetrical (Fig. 2 a). However, with the larger cations there is some evidence of rectification with single-channel currents at negative holding potentials (current flow luminal to cytosolic) being slightly larger than those at positive holding potentials (current flow cytosolic to luminal). For example, the currents at 80 and -80 mV for methylamine, trimethylamine and methyldiethanolamine are respectively 22.7 ± 1.1 and -23.4 ± 0.72, 4.3 ± 0.2 and -5.0 ± 0.2, and 1.39 ± 0.09 and -1.63 ± 0.07 pA (n = 4 except the first figure which is n = 3; ±SEM except the first figure which is ±SD); the current at the positive holding potential is respectively, 97, 86, and 85% of that at the negative holding potential. Given the small quantitative nature of this rectification, values quoted in Table 1 for conductances are determined from linear regression of the current-voltage relationship over the entire voltage range examined.

<table>
<thead>
<tr>
<th>Name and formula</th>
<th>( E_{rev} ) ± SEM ( mV )</th>
<th>( P_L/P_H )</th>
<th>G ± SEM ( pS )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia; ( \text{NH}_4^+ )</td>
<td>1.7 ± 0.2</td>
<td>1.42 ± 0.3</td>
<td>594 ± 4</td>
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<td>Hydrazine; ( \text{NH}_2\text{NH}_2^+ )</td>
<td>1.8 ± 1.2</td>
<td>0.95 ± 0.4</td>
<td>389 ± 9</td>
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<td>Methylamine; ( \text{CH}_3\text{NH}_2^+ )</td>
<td>1.91 ± 0.5</td>
<td>0.67 ± 0.05</td>
<td>286 ± 5</td>
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<td>Dimethylamine; ( \text{CH}_3\text{CH}_2\text{NH}_2^+ )</td>
<td>2.3 ± 0.3</td>
<td>0.32 ± 0.04</td>
<td>132 ± 4</td>
</tr>
<tr>
<td>Trimethylamine; ( \text{CH}_3\text{CH}_3\text{NH}^+ )</td>
<td>2.8 ± 0.4</td>
<td>0.14 ± 0.04</td>
<td>59.0 ± 2</td>
</tr>
<tr>
<td>Ethylamine; ( \text{C}_2\text{H}_5\text{NH}_2^+ )</td>
<td>2.2 ± 6</td>
<td>0.51 ± 4</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>Propylamine; ( \text{C}_3\text{H}_7\text{NH}_2^+ )</td>
<td>2.3 ± 0.6</td>
<td>0.42 ± 0.04</td>
<td>37.0 ± 1.4</td>
</tr>
<tr>
<td>Isopropylamine; ( \text{CH}_2\text{CH}_2\text{NH}_2^+ )</td>
<td>2.9 ± 0.7</td>
<td>0.37 ± 0.04</td>
<td>41.3 ± 0.9</td>
</tr>
<tr>
<td>Dimethylmethyamine; ( \text{C}_2\text{H}_5\text{C}_2\text{H}_3\text{NH}^+ )</td>
<td>2.9 ± 0.5</td>
<td>0.14 ± 0.04</td>
<td>22.0 ± 0.2</td>
</tr>
<tr>
<td>Diethylmethyamine; ( \text{C}_2\text{H}_5\text{CH}_2\text{NH}^+ )</td>
<td>3.1 ± 1.2</td>
<td>0.09 ± 0.04</td>
<td>10.4 ± 0.6</td>
</tr>
<tr>
<td>Diethylamine; ( \text{C}_2\text{H}_5\text{NH}^+ )</td>
<td>2.5 ± 0.5</td>
<td>0.24 ± 0.04</td>
<td>19.4 ± 0.4</td>
</tr>
<tr>
<td>Ethanolamine; ( \text{C}_2\text{H}_5\text{OHNH}_2^+ )</td>
<td>2.3 ± 0.6</td>
<td>0.48 ± 0.04</td>
<td>125 ± 1.0</td>
</tr>
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<td>Ethylethanolamine; ( \text{C}_2\text{H}_5\text{CH}_2\text{OHNH}_2^+ )</td>
<td>2.6 ± 0.6</td>
<td>0.22 ± 0.04</td>
<td>23.0 ± 0.1</td>
</tr>
<tr>
<td>Propanolamine; ( \text{C}_3\text{H}_7\text{OHNH}_2^+ )</td>
<td>2.3 ± 0.2</td>
<td>0.35 ± 0.04</td>
<td>55.3 ± 0.8</td>
</tr>
<tr>
<td>Diethanolamine; ( \text{C}_2\text{H}_4\text{OHNH}_2^+ )</td>
<td>2.8 ± 0.8</td>
<td>0.20 ± 0.04</td>
<td>31.0 ± 1.0</td>
</tr>
<tr>
<td>Methyldiethanolamine; ( \text{CH}_3\text{OHNH}_2^+ )</td>
<td>2.3 ± 0.4</td>
<td>0.25 ± 0.04</td>
<td>62.2 ± 0.7</td>
</tr>
<tr>
<td>Diethylethanolamine; ( \text{C}_2\text{H}_4\text{OCH}_2\text{NH}^+ )</td>
<td>2.9 ± 0.9</td>
<td>0.14 ± 0.04</td>
<td>29.5 ± 0.8</td>
</tr>
<tr>
<td>Methyldiethanolamine; ( \text{CH}_3\text{OCH}_2\text{NH}_2^+ )</td>
<td>2.9 ± 0.5</td>
<td>0.10 ± 0.04</td>
<td>14.6 ± 0.1</td>
</tr>
<tr>
<td>Triethanolamine; ( \text{C}_3\text{H}_7\text{OHNH}_3^+ )</td>
<td>3.6 ± 0.4</td>
<td>≥ 80 mV</td>
<td>≤ 0.04 ± 0.04</td>
</tr>
<tr>
<td>Triethylamine; ( \text{C}_3\text{H}_7\text{NH}_3^+ )</td>
<td>3.6 ± 2</td>
<td>≥ 80 mV</td>
<td>≤ 0.04 ± 0.04</td>
</tr>
<tr>
<td>Diethylethanolamine; ( \text{C}_2\text{H}_5\text{CH}_2\text{OHNH}_2^+ )</td>
<td>3.6 ± 2</td>
<td>≥ 80 mV</td>
<td>≤ 0.04 ± 0.04</td>
</tr>
<tr>
<td>Ethylethanolamine; ( \text{C}_2\text{H}_5\text{CH}_2\text{OHNH}_2^+ )</td>
<td>3.5 ± 4</td>
<td>≥ 80 mV</td>
<td>≤ 0.04 ± 0.04</td>
</tr>
<tr>
<td>l-Lysine; ( \text{CHO-NH}^+ ) ( \text{CH}_3\text{CH}_2\text{NH}_2^+ )</td>
<td>3.2 ± 3</td>
<td>≥ 80 mV</td>
<td>≤ 0.04 ± 0.04</td>
</tr>
</tbody>
</table>

Conditions and methods are as indicated in the text. G is the single-channel conductance in symmetrical 210 mM cation. The other abbreviations are as in the text.
Organic Cation Relative Permeability

The reversal potential ($E_{\text{rev}}$) under bi-ionic conditions, with 210 mM K⁺ at the luminal face of the channel and 210 mM test cation at the cytoplasmic face, was measured in a series of experiments. Fig. 2b shows sample current-voltage relationships and Table I gives the $E_{\text{rev}}$ together with relevant statistics and the calculated permeability ratio. Triethylamine, triethanolamine, L-lysine, diethylethanolamine and ethyldiethanolamine were effectively nonpermeant with reversal potentials equal to or in excess of 80 mV under bi-ionic conditions as above.

For two ions the reversal potential was also determined with 210 mM K⁺ at the cytoplasmic face of the channel and the test cation at the luminal face of the channel. For methylamine the calculated $E_{\text{rev}}$ was $-13.2 \pm 1.6$ mV and for the larger trimethylamine it was $-59.3 \pm 2.5$ mV ($n = 3$; $\pm SD$; and both corrected for junction potentials). Thus, there does seem to be some difference in the measured reversal potential and thus relative permeability given the orientation of the solutions about the channel.

If it is intended to assign a physical interpretation to the permeability ratios, then it is useful to measure the behavior of the reversal potential with varying test cation concentration. It is an issue essentially of ion occupancy: in an ion channel where more than one ion is able to occupy the conduction pathway, permeability ratios may be concentration-dependent (Hille and Schwarz, 1978). In such multi-ion channels relative permeability may be determined by variations in occupancy and ion-ion repulsion as much as by physical factors relating to the permeant ion. Consequently we have examined the concentration dependence of permeability ratios in two ways.

In the first, the variation of reversal potential with increasing ethanolamine concentration at the cytoplasmic face of the channel was examined whilst the concentration of K⁺, equivalent to the luminal concentration, was maintained constant at 210 mM. The behavior is essentially Nernstian with a plot of $E_{\text{rev}}$ against $\ln [\text{Ethanolamine}]/[K^+]$ (Fig. 3a) a straight line ($r = -1.0$) with a slope of $-24.5 \pm 0.5$ mV ($\pm SEM$) and
a y intercept of 19.1 mV, i.e., when the ethanolamine and K⁺ concentration are equal, giving a permeability ratio of 0.47 close to the figure in Table I. The slope is not significantly different from the expected value of -25.2 mV (the value of RT/F at 20°C). A second related experimental test is to examine the concentration-dependence of the reversal potential in which the ratio of permeant cation activity under bi-ionic conditions is kept constant whilst the absolute value is varied. This relationship was explored with varying K⁺ activities at the luminal face of the channel and matched dimethylamine activities at the cytosolic face. Fig. 3 b shows that the reversal potential is essentially concentration-independent. Thus, the permeability process that discriminates monovalent cations such as K⁺ from the organic cations is Nernstian and concentration-independent. These results are compatible with our assertion that permeant ion conduction in the sheep cardiac SR Ca²⁺-release channel is largely consistent with single-ion occupancy (Tinker et al., 1992a) and in particular

![Fig. 3](image)

**Figure 3.** (A) A plot of the K⁺/ethanolamine reversal potential against Ln([Ethanolamine]/[K⁺]). The data points are indicated by the squares and the SEM is included within the symbol. The line is a linear regression fit with parameters as given in the text. (B) A plot of the K⁺/dimethylamine reversal potential against K⁺ concentration. The line is drawn on the assumption that the K⁺/dimethylamine reversal potential is concentration independent and is equal to 29.8 mV. The data points are indicated by the squares and the SEM is included within the symbol.

complements our previous observation that the monovalent-divalent permeability ratio is concentration-independent (Tinker and Williams, 1992). Indeed, within experimental error the Ca²⁺-K⁺ permeability ratio is independent of concentration even under asymmetrical ionic conditions and also under those approaching the situation supposedly found in the intact myocyte (Tinker et al., 1992a; Tinker et al., 1993).

**Factors Determining Single-Channel Conductance**

The data presented in Table I indicate that there is a general tendency for larger ions to have a lower relative permeability and lower conductance. However a closer inspection reveals that permeant cations with similar permeability relative to K⁺ can have different conductances. For example, compare the group of cations trimethylamine, ethyldimethylamine and also the group diethylmethylamine and diethyl-
amine, diethanolamine and ethylethanolamine. The other determinant of ion selectivity is the relative affinity of the conduction pathway for different permeant ions. There are two methods of examining this experimentally. The first is to examine the variation of single-channel conductance with increasing permeant organic cation activity. However this suffers from the theoretical problem that at low permeant ion activity surface charge may significantly influence the relationship and its unambiguous interpretation in terms of ion binding. Practically, it may also be dependent on the very accurate measurement of small changes in single-channel conductance with permeant ions, whose conductance in the Ca$^{2+}$-release channel is already low. As a result we have adopted a different approach in which the decrease in single-channel current in symmetrical 210 mM K$^+$ is measured at a fixed holding potential consequent upon the addition of increasing concentrations of organic cations. If it is assumed that there is simple competition in binding to the channel between K$^+$ and the permeant organic cation then as the permeant organic cation concentration is increased symmetrically the single-channel current should decrease as described by an equation of the kind

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

(2)

where $[X^+]$ is the organic cation concentration, $I_c$ is the control current in 210 mM K$^+$ at the fixed holding potential, $I$ is the current in the presence of the organic cation, $r_{\text{limit}}$ is determined by the saturating current of the organic cation and $K_D$ is a measure of the relative affinity (relative to K$^+$ in this case) of the organic cation for the conduction pathway. Using this approach it is possible to examine any difference in relative affinity between two organic cations that may explain differences in conductance.

We have performed such experiments at a holding potential of 60 mV with the cations trimethylamine and diethylmethylamine. Fig. 4 shows representative single-channel current traces and Fig. 5 the relationship of $(1 - I/I_c)$ to permeant ion...
blocker concentration for both ions. The best fit parameters given by nonlinear regression for the block generated by trimethylamine are $K_D = 55.4 \pm 3.1$ mM and $r_{\text{limit}} = 0.92 \pm 0.02$ and for the block generated by diethylmethylamine they are $K_D = 20.8 \pm 1.4$ mM and $r_{\text{limit}} = 0.98 \pm 0.03$. The Hill coefficients of 0.91 and 1.0 respectively determined from Hill plots of the data make it likely that both these ions are interacting with a single site in a strictly competitive fashion with little evidence of positive or negative cooperativity (data not shown). Thus, there is a significant difference in affinity with diethylmethylamine having an almost threefold higher affinity than trimethylamine. This and the slightly lower permeability ratio give adequate reasons for the approximately fivefold lower single-channel conductance seen with diethylmethylamine compared to trimethylamine. Interestingly with both ions the effect is much less pronounced at negative holding potentials. This is in contrast to permeant ion block seen with divalent cations which is equally pronounced at positive and negative holding potentials (Tinker et al., 1992a). A possible explanation for this phenomena is offered below.

### Locating the Selectivity Filter in the Electrical Field

The organic cation triethylamine is unable to act as a current carrier in the sheep cardiac SR Ca$^{2+}$-release channel but it does have an effect on K$^+$ conductance when added to the cytosolic but not the luminal face of the channel. The addition of 25 mM triethylamine leads to a reduction in single-channel current amplitude which is more pronounced at positive than negative holding potentials. This is illustrated in Fig. 6, a and b. The block is thus voltage-dependent and can be investigated by the quantitative approach first used by Woodhull (1973). In the simplest form of this analysis there is a single site, accessible to a blocker of valence $z$ from only one side of the channel, lying a fraction $\delta$ into the voltage drop. The expression for the change in relative chord conductance ($I/I_{0}$, the current in the presence of blocker divided by the control current) with variations in voltage ($V$) and blocker concentration ($[B]$) is

$$\frac{I}{I_0} = \frac{1}{1 + \frac{[B]}{K_D(0)} e^{zF(V/R)T}}$$

Figure 5. The reduction in relative conductance ($1 - I/I_c$) at a holding potential of 0 mV with increasing trimethylamine (■) and diethylmethylyamine (▲) concentration. The two solid lines are the best fit rectangular hyperbola with parameters as indicated in the text. The SEM is included within the symbol or indicated by the error bars.
where $K_b(0)$ is the dissociation constant at zero mV holding potential and $\delta$ is
customarily referred to as the effective valence. $F$, $R$, and $T$ have their usual
meanings and $RT/F$ is 25.2 mV at 20°C.

The change in relative conductance with holding potential was studied in four
experiments after the addition of 25 mM triethylamine. Despite the effect only being
apparent on addition of the organic cation to the cytosolic face of the channel,

\begin{figure}
\centering
\includegraphics[width=0.7\textwidth]{figure6}
\caption{(A) Representative single-channel current fluctuations recorded at holding poten-
tials of ±60 mV in symmetrical 210 mM K+ with and without triethylamine added symmetri-
cally as indicated in the diagram. The data were filtered at 1 kHz and digitized at 1 kHz. The
dotted lines indicate the current amplitude of the open state. (B) An example of current-
voltage relationships recorded in symmetrical 210 mM K+ before (□) and after the addition of
symmetrical 25 mM triethylamine (■). The linear regression line ($r = 1.0$) through the control
points is equivalent to a slope conductance of 728 pS. (C) The behavior of relative conductance
with holding potential in symmetrical 210 mM K+ with symmetrical 25 mM triethylamine. The
data points are indicated by the squares and the SEM is indicated by the error bars or included
within the symbol if not. The line is the best fit of Eq. 3 to the data and has parameters as
indicated in the text.}
\end{figure}

experiments were performed after symmetrical addition to avoid asymmetric surface
potentials developing (Miller, 1982a). Nonlinear regression was used to fit Eq. 3 to
the mean data from four experiments (Fig. 6 c). The best fit parameters were $\delta =
0.82 \pm 0.03$ and $K_b(0) = 77.4 \pm 3.6$ mM $(\pm$SEM). This figure agrees with our previous
studies on the small tetraalkyl ammonium cations (excepting tetramethyl ammon-
nium) and the charged local anaesthetics QX222 and Procaine (Tinker et al., 1992c;
Tinker and Williams, 1993) which also cause voltage-dependent block with an effective valence of ~0.9. The data suggest that for impermeant organic cations the narrowest region of the pore occurs over the last 10% of the voltage drop at the luminal face of the channel.

If similar experiments are performed with triethanolamine, relative conductance appears to reach a limiting value above a holding potential of 60 mV and increasingly deviates from a Woodhull type scheme with an effective valence of 0.8 to 0.9 (data not shown). This behavior might be expected if triethanolamine is actually able to permeate the sheep cardiac Ca\(^{2+}\)-release channel at high potentials leading to relief of block.

**Permeant Ion Block and Modeling such Interactions**

The addition of permeant organic cations symmetrically also has an effect on K\(^+\) conductance in the Ca\(^{2+}\)-release channel. Fig. 7 b shows the effects of adding 60 mM trimethylamine and 25 mM methyldeethylamine to channels bathed symmetrically in 210 mM K\(^+\). Qualitatively, a similar kind of effect is obtained as with the impermeant triethylamine with currents reduced more at positive than negative holding potentials for a given concentration. However a quantitative approach to such problems is more difficult than for impermeant ion block. One method is to model ionic conduction using Eyring rate theory. We have given evidence that ionic conduction in the sheep cardiac SR Ca\(^{2+}\)-release channel proceeds in a single-file fashion with at most one ion able to occupy the conduction pathway. Computer modeling based on this hypothesis has led us to develop a minimal generalized energy profile shown in Fig. 7 a. We have tested a specific set of energy profiles for monovalent and divalent cations against the available conduction data and find that it produces reasonable quantitative agreement (Tinker et al., 1992a). Broadly, it is envisaged that an ion traverses four major energy peaks and binds to three binding sites on its passage through the channel. Models based on fewer peaks/wells are unable to reproduce satisfactorily the experimental data set. Is it possible to extend such a treatment to the organic cations trimethylamine and diethylmethylamine? Table II shows the energy profiles tested together with the energy profile previously determined for K\(^+\).

To model organic cation conduction several changes in the energy profile were necessary. The energy peaks encountered by an organic cation, in comparison with a permeant group Ia cation such as K\(^+\), were assumed to be increased throughout the electrical field but in particular in crossing peak 4. Secondly, binding was assumed to be increased in affinity at wells 1 and 3, especially at the latter, whilst binding at the central well was unaffected. These changes are not unreasonable given that the impermeant organic cations do not appear to be able to cross the last 10% of the voltage drop and that the \(K_b(0)\) for such blockers decreases with increasing hydrophobicity (Tinker et al., 1992c).

The predicted single-channel conductance in symmetrical 210 mM solutions are 53 (the experimentally observed figure is 59) and 9.6 (the experimentally observed figure is 10.4) pS and the predicted permeabilities are 0.17 (the experimentally observed figure is 0.14) and 0.09 (the experimentally observed figure is 0.09) (210 mM organic cation at the cytoplasmic and 210 mM K\(^+\) at the luminal face of the channel) for trimethylamine and diethylmethylamine respectively. A degree of
FIGURE 7. (A) A diagrammatic representation of the voltage- and concentration-independent energy profile used to model ion translocation in the sheep cardiac SR Ca\textsuperscript{2+}-release channel. The bar indicates the position of the energy wells in the electrical field. The rationale for choosing such a profile is given in (Tinker, Lindsay, and Williams, 1992a) and the individual parameters for the ions examined in Table II. (B) The single-channel current-voltage relationship in symmetrical 210 mM K\textsuperscript{+} after the addition of 60 (■), 100 (■), and 200 (▲) mM trimethylamine added to both chambers. The points are the mean of four experiments with standard error bars included within the symbol. The associated curves are the predictions from the modeling work using energy profiles as indicated in Fig. 7A. (C) The single-channel current-voltage relationship in symmetrical 210 mM K\textsuperscript{+} after the addition of 25 mM diethylmethylamine added to both chambers. The points are indicated by the squares and are the mean of four experiments with standard error bars included within the symbol. The associated curve is the prediction from the modeling work using energy profiles as indicated in Table II.

TABLE II

The Voltage and Concentration Independent Energy Profiles (in Multiples of RT) Used to Model Organic Cation Conduction

<table>
<thead>
<tr>
<th>Cation</th>
<th>Peak 1</th>
<th>Peak 2</th>
<th>Peak 3</th>
<th>Peak 4</th>
<th>Well 1</th>
<th>C Well</th>
<th>Well 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TriMet</td>
<td>6.75</td>
<td>6.75</td>
<td>6.75</td>
<td>8.80</td>
<td>−2.85</td>
<td>−3.25</td>
<td>−3.15</td>
</tr>
<tr>
<td>DiEMet</td>
<td>6.75</td>
<td>6.75</td>
<td>6.75</td>
<td>10.55</td>
<td>−3.15</td>
<td>−3.25</td>
<td>−3.45</td>
</tr>
<tr>
<td>K\textsuperscript{+}</td>
<td>5.50</td>
<td>5.50</td>
<td>5.50</td>
<td>5.50</td>
<td>−2.35</td>
<td>−3.25</td>
<td>−2.35</td>
</tr>
</tbody>
</table>

TriMet stands for trimethylamine and DiEMet for diethylmethylamine.
rectification is seen in the predicted current-voltage relationships with current at a holding potential of 80 mV being 84 and 76% of that at -80 mV for trimethylamine and diethylmethylamine respectively.

The energy profiles for the examined organic cations then give a reasonable approximation to the observed conduction properties but how well do they predict permeant ion block? Fig. 7, b and c show the observed and predicted current-voltage relationships in symmetrical 210 mM K+ when a series of trimethylamine concentrations are added symmetrically (Fig. 7 b) and when 25 mM diethylmethylamine is added symmetrically (Fig. 7 c). Once again the quantitative agreement is reasonable.

**DISCUSSION**

**Physical Factors Involved in Determining Relative Permeability**

One rationale for performing studies such as those described here is to examine the relationship between selectivity and permeating ion structure and hence to deduce structural motifs in the conduction pathway of the channel under study. We have previously argued that there is little evidence for ion-ion interactions during ion permeation in the sheep cardiac SR Ca2+ release channel (Williams, 1992) and ionic conduction is reasonably approximated by single-ion models. The concentration independence of the reversal potential seen with the organic cations adds support to this tenet. In such paradigms the permeability ratio is dependent only on differences in peak heights in the energy profiles and is uninfluenced by well depth (Läuger, 1973). Are there any clues from Table I as to how the permeability differences amongst the organic cations can be explained? Fig. 8 a shows the behavior of relative permeability, as measured by the reversal potential under bi-ionic conditions, for various types of substitution around the NH4+ nucleus. Substituting methyl groups around the nitrogen leads to a more pronounced increase in reversal potential, i.e., a steep decline in relative permeability (the left panel in Fig. 8 a) whilst in contrast extending the length of a side chain only leads to an attenuated effect on reversal potential (the right panel in Fig. 8 a). By constructing computer energy minimized CPK equivalent models (see Methods) the relationship of permeant ion structure to relative permeability could be investigated. Examples of two-dimensional silhouettes of such energy minimized models for trimethylamine and propylamine are shown in two orientations in Fig. 8 b. The “minimal” orientation corresponds approximately to the profile with minimum surface area and the “maximal” to that with maximum surface area. It makes the point that it is possible to orientate these two ions of similar total volume or bulk so that propylamine may present a significantly smaller area to the narrowest portion of the channel. It offers an appealing hypothesis to explain the observations in Fig. 8 a.

Consequently we have put such ideas on a quantitative footing by measuring the minimum circular radius in which an ion can be surrounded and this correlates inversely with permeability as shown in Fig. 8 c. It explains why derivatives in which the organic chain is lengthened, rather than derivatives with methyl group substitution around the NH4+ nucleus, show a slower decline in relative permeability; the minimum circular radius in which the ion can be enclosed in the former group
Figure 8. (A) The left panel shows the change of reversal potential following the addition of methyl groups around an ammonium nucleus. The data points are indicated by squares (n as given in Table I and ±SEM) and the curve is of no theoretical significance. The right panel shows the change of reversal potential after the lengthening of a side chain by the addition of CH2/CH3 groups around an ammonium nucleus. The data points are indicated by triangles (n as given in Table I and ±SEM) and the curve is of no theoretical significance. (B) The silhouettes of energy minimised CPK equivalent models for the cations trimethylamine and propylamine. The two different orientations of each ion are explained in the text. Each ion is rotated through approximately 180° between the minimal and maximal view. (C) A plot of relative permeability against cation radius. The solid line indicates the predictions of simple excluded area theory (Eq. 4) with parameters as given in the text, the long dashed line the predictions of excluded area theory with a simple term for solute-wall friction (Eq. 5) with parameters as given in the text and the short dashed line a more complex expression including a term for cation/channel wall friction with parameters as indicated in the text. The latter expression is given by Eq. 5 multiplied by

\[
\frac{(1 - 2.105 \times X + 2.0865 \times X^3 - 1.7068 \times X^5 + 0.72603 \times X^6)}{(1 - 2.105 \times Y + 2.0865 \times Y^3 - 1.7068 \times Y^5 + 0.72603 \times Y^6)} \times (1 - 0.75857 \times (X^5) \times (1 - 0.75857 \times Y^6))
\]

where \(X = R_{X+/RC}\) and \(Y = R_{K+/RC}\). The inset shows the linearization of the excluded area relationship suggested by (Cohen, Labarca, Davidson, and Lester, 1992). Calculating the \(RC\) and \(R_{K+}\), as indicated in that paper gives figures of 3.63 and 1.67 Å, respectively (\(r = -0.91\)).
increases less steeply with each methyl group substitution. In addition, the substitution of a hydroxyl for a hydrogen in the side chain had little effect on relative permeability; for example compare diethylamine, diethanolamine and ethylethanolamine in Table I. The process approximates to simple sieving given that the ions orientate themselves in a particular fashion and specific chemical factors such as hydrogen bonding are not a major determinant of permeability.

It is possible to carry out a further quantitative test of this hypothesis. One such theoretical formalism is the excluded area hypothesis in which permeability is determined by the squared difference in permeant ion radius and the minimum channel radius encountered by the ion.

It can formally be stated as

$$
\frac{P_{X^+}}{P_{K^+}} \propto \frac{(R_C - R_{X^+})^2}{(R_C - R_{K^+})^2}
$$

where \( R_{K^+} \), \( R_C \) and \( R_{X^+} \) are the permeating \( K^+ \) cation radius, the radius of the narrowest region of the channel and the radius of the cation \( X^+ \). Extensions of this equation to include an expression for frictional drag between the permeating ion, solute and the channel wall are possible (Renkin, 1955; Soloman, 1968; Dwyer et al., 1980; Cohen et al., 1992b). One of the simplest is

$$
\frac{P_{X^+}}{P_{K^+}} \propto \frac{(R_C - R_{X^+})^2}{(R_C - R_{K^+})^2} \frac{(R_C - 2.4R_{K^+})}{(R_C - 2.4R_{X^+})}
$$

which takes account, in a simple fashion, of solute-wall drag. Higher power terms of the ion to channel radius are available to further describe the influence of solute-wall friction on hydrodynamic drag; one such approximation is given in the legend to Fig. 8c.

The use of such approaches is illustrated in Fig. 8c for the tested ions. It can be seen that size is inversely related to relative permeability. Eq. 4 (shown as the solid line in Fig. 8c) and 5 (shown as the long dashed line in Fig. 8c) were fitted by nonlinear regression to the data and the channel radius and the effective permeating radius for \( K^+ \) were allowed to vary freely. The channel radii obtained were 3.29 ± 0.14 and 3.46 ± 0.17 Å (±SEM) whilst the effective permeating radii for \( K^+ \) are 1.79 ± 0.06 and 1.79 ± 0.04 Å (±SEM), respectively. For comparison a more complex expression taking account of solute-wall friction is shown with the channel radius fixed at 3.5 Å and an effective permeating radius of 1.8 Å for \( K^+ \) (shown as the short dashed line in Fig. 8c). All the approaches give a reasonable description of the data. On balance it seems likely that the channel minimum radius is between 3.3 and 3.5 Å. This is consistent with the dimensions of the effectively impermeant organic cations given in Table I. Solute-wall friction appears not to be a major factor as lengthening a side chain has little effect on relative permeability (Fig. 8a) and the relative permeability does not decline as steeply as would be predicted theoretically with increasing ionic radius (Fig. 8c).

However, such estimates should be critically interpreted. The assumption that the selectivity filter is circular is arbitrary and other geometries are possible. Continuum theories, in which ions are simply viewed as spheres moving through a viscous
medium and channels approximated as pipes, may not be appropriate for the
description of events at the molecular level (Levitt, 1973). In addition, channel
proteins may not be as rigid as is implicit in such treatments and may show some
degree of "give" when allowing large ions to permeate. In the subsequent discussion
this narrow portion of the channel will be referred to as the selectivity filter.

Whilst the use of excluded area theory or variations on it are largely successful,
there are examples of some permeant ions in the sheep cardiac SR Ca$^{2+}$-release
channel in which simple sieving is not the only determinant of relative permeability.
Isopropylamine is one such example in which the relative permeability is higher than
would be expected on the above theoretical grounds. In addition, we have previously
examined the interactions of Tris$^+$ and tetramethyl ammonium (TMA$^+$) cations with
the conduction pathway. Whilst Tris$^+$ is permeant ($P_{\text{Tris}}^+/P_{\text{K}}^+ = 0.20$, single channel
conductance in symmetrical 210 mM salt = 17.4 pS) TMA$^+$ is effectively impermeant
(Lindsay et al., 1991; Tinker et al., 1992c) and yet Tris$^+$ is the larger cation in all
orientations. In addition with Tris$^+$ we were forced to use a slightly different energy
profile than those used in Fig. 7 a to explain ion conduction (Tinker et al., 1992a).

Cation sieving is clearly not at work in determining the relative permeability of the
group Ia and alkaline earth divalent cations in the cardiac SR Ca$^{2+}$-release channel;
there are only very minor differences between members of these two groups. Even
given these small differences in relative permeability, neither crystal nor hydrated
cation radius seems to be related to relative permeability (Lindsay et al., 1991; Tinker
and Williams, 1992).

**Permeant and Impermeant Ion Block**

Do we know anything of the location of the selectivity filter in the electrical field?
Impermeant ions such as triethylamine, the effects of which have been described in
this paper, together with the small tetraalkyl ammonium cations and the local
anaesthetics QX222 and Procaine (Tinker et al., 1992c; Tinker and Williams, 1993),
produce a voltage-dependent block with an effective valence of 0.8 to 0.9. The
simplest interpretation of such studies is that these ions are interacting with a
single-site located 90% of the way across the voltage drop from the cytoplasmic face
of the channel. If electrical distance is proportional to physical distance then the
narrowest part of the conduction pathway is located close to the lumen of the
sarcoplasmic reticulum.

Permeant ions, such as trimethylamine and diethylmethylamine, are also able to
inhibit K$^+$ current. We have previously performed similar types of experiments using
the divalent cations as permeant channel blockers. Whilst those ions reduced current
approximately equally at positive and negative holding potentials; the behaviour is
strikingly different with the permeant organic cations. They lead to significant
rectification which is qualitatively similar to that seen with the impermeant organic
cations. As explained previously, a different quantitative approach is necessary to
interpret the effects of these permeant organic cations on K$^+$ conductance. We have
used a single-ion four peak, three binding site Eyring rate theory model developed
from our previous work (Tinker et al., 1992a) in an attempt to simulate these
interactions. Such models, as demonstrated in the results, are able to give a
reasonable quantitative description of the data. In terms of the model, two essential
features account for the complex conduction properties; a peak located at the luminal end of the channel (peak 4) and a binding site (well 3) just prior to it. To “fine tune” quantitative agreement with the data it is also necessary to postulate an increase in affinity at a site presumed to be encountered by a cation on entering the voltage drop from the cytoplasmic face of the channel and an increase in height at the three other peaks in the channel relative to those experienced by K⁺. As the permeant ion increases in size, peak 4 becomes higher and well 3 deeper, leading to a decline in single-channel conductance. In addition, an organic ion will become impermeant when its size is such that peak 4 is effectively insurmountable. Thus, though the two approaches to explaining permeant and impermeant ion block are different, it is possible to see intuitively that one may come to approach the other.

Several observations support the likelihood that the binding site, well 3 in the model, is largely hydrophobic. The apparent Kₒ for the permeant ion diethylmethylamine is higher than that for trimethylamine. In general there is a trend such that increasing side chain length produces little change in permeability but a significant fall in conductance. An analogous observation is that if ethyl or propyl groups are substituted with ethanol or propanol groups the permeability changes little but the conductance often rises. It is likely that these conductance changes primarily reflect increases and decreases in affinity at well 3 consequent upon varying relative hydrophobicity of the interacting ion. The Kₒ(0) of a series of blockers decreases as the number of methyl groups increases (Tinker et al., 1992c) and triethylamine is compatible with this trend.

Comparisons with Other Isoforms and Ion Channels

The Ca²⁺-release channel exists as two isoforms, one present in skeletal muscle and the other in cardiac and nervous tissue, with approximately 66% homology in primary structure (Otsu et al., 1990). The putative transmembrane segments are largely conserved between the two isoforms. The available conduction data regarding the skeletal isoform are more limited. Other authors (Smith et al., 1988) have reported that organic cations as large as choline (minimum circular radius ~ 2.9 Å) are permeant in the rabbit skeletal SR Ca²⁺-release channel with a single-channel conductance of ~ 20 pS. A related observation is that there is a significant flux of sugars for example xylose (minimum circular radius ~ 3.4 Å) through the rabbit skeletal SR Ca²⁺-release channel, as measured by tracer or light scattering methods (Meissner, 1986; Kasai, Kawasaki, and Yamamoto, 1992). Both these sets of experimental findings are compatible with a wide permeation pathway of a similar order of magnitude as the cardiac isoform for the rabbit skeletal channel.

The sheep cardiac SR Ca²⁺-release channel is much less selective than the voltage-activated Na⁺ channel and a wide variety of K⁺ channels; it shows little discrimination in terms of relative permeability amongst monovalent or divalent cations (Tinker et al., 1992a). The minimum pore radius of ~ 3.5 Å is much larger than that found for the voltage-dependent Na⁺ and K⁺ channels and is in keeping with ions passing through the SR Ca²⁺-release channel in a hydrated fashion with the inner waters of hydration remaining relatively intact. The dihydropyridine receptor in skeletal muscle has also been estimated to have a relatively large pore size (~ 6.0-Å diam) (McCleskey and Almers, 1985); however, there are many differences in other
conduction properties from the SR Ca$^{2+}$-release channel most notably that the
divalent/monovalent permeability ratio is orders of magnitude higher in the dihy-
dropyridine receptor and that monovalent current can be inhibited by μM quantities
of Ca$^{2+}$ (Tsien, Hess, McCleskey, and Rosenberg, 1987) as opposed to mM quantities
in the SR Ca$^{2+}$-release channel. The selectivity profile of the SR Ca$^{2+}$-release channel
most resembles those of a disparate grouping of channels (Hille, 1992) from a variety
of tissues including the NMDA receptor of the mammalian central nervous system
(Mayer and Westbrook, 1987), cGMP-activated channels from vertebrate retinal rods
(McNaughton, 1990), 5-Hydroxytryptamine (5-HT)-gated channels in neuroblastoma
cells (Yang, 1990) and the nicotinic acetylcholine receptor (Adams, Dwyer, and Hille,
1980; Dani and Eisenman, 1987). All these channels display little difference in
relative permeability amongst the group Ia monovalent cations or the alkaline earth
divalent cations and there is measurable permeability to even quite large organic
cations leading to estimates of pore radii of 2.5 to 3.8 Å (Dwyer et al., 1980; Picco
and Menini, 1993). The relative divalent to monovalent permeability varies from
~0.2 for the nicotinic acetylcholine receptor up to figures of approximately 10.0 for
the NMDA receptor; close to the values we have determined for the ryanodine
receptor (Mayer and Westbrook, 1987; Tinker and Williams, 1992). Studies of ion
permeation in the nicotinic acetylcholine receptor have now progressed to the
molecular level with the identification of the possible short sequence of amino acids
in the M2 region in each of the subunits responsible for the narrowing of the
selectivity filter (Lester, 1992; Cohen, Labarca, Czyzyk, Davidson, and Lester, 1992).
The observation that some of the proposed transmembrane segments of the cloned
skeletal SR Ca$^{2+}$-release channel do show limited homology with the M2 segment of
the nicotinic acetylcholine receptor is tantalising (Takeshima et al., 1989).

One major difference between the channels discussed above is the single-channel
conductance measured in comparable conditions in monovalent cations: it varies
from 0.5 pS for the 5-HT-activated channel through to ~50 pS for the NMDA
receptor, to a massive 720 pS for the SR Ca$^{2+}$-release channel. One possible
explanation could be if single-channel conductance were significantly influenced by
electrodiffusion in wide vestibule regions; the size and electrostatics of which varies
amongst the above channels. The SR cardiac Ca$^{2+}$-release channel has a distinctive
and unique “finger-print” for ion selectivity. It is of considerable physiological
interest as to whether this extends to the skeletal isoform and even to the IP$_3$
receptor-channel, forming a related family of intracellular channels involved in
Ca$^{2+}$-release for signaling. Of interest in this context is the recently reported
observation that the IP$_3$ receptor-channel of canine cerebellum is permeable to a
range of divalent cations (Bezprozvanny and Ehrlich, 1993). The relative conduc-
tance sequence of these cations in the IP$_3$ receptor-channel (Ba$^{2+}$ > Sr$^{2+}$ > Ca$^{2+}$
> Mg$^{2+}$) is the same as that reported by us for the cardiac ryanodine receptor-channel
(Tinker and Williams, 1992).

There is a subtle difference between our studies and those performed on the
nicotinic acetylcholine receptor. In the latter work a mean radius is calculated from
the volume of the permeating ion whilst our quoted radius is the minimal circular two
dimensional radius in which the ion can be enclosed after rotating it in three
dimensions; the observations in Fig. 8 a are better explained when related to the
latter measurement. This implies that in order to permeate the sheep cardiac SR Ca\(^{2+}\)-release channel some organic cations must strike the selectivity filter in a particular range of orientations. This is not totally inconceivable: the geometry of the conduction pathway before the selectivity filter may constrain ions to certain arrangements such that the minimum circular area of the permeating ion is presented to the narrowest region of the pore. Similar kinds of argument, together with the possibility of hydrogen bonding, have been used to explain the permeation of aminoguanidine in the sodium channel and the permeation and block of organic cations in the nicotinic acetylcholine receptor (Hille, 1992).

In conclusion, these studies indicate that the permeation of charged organic cations in the sheep cardiac sarcoplasmic reticulum Ca\(^{2+}\)-release channel is compatible with sieving through a narrowing of minimum radius 3.3–3.5 Å located in the 10–20% of the voltage drop closest to the luminal face of the channel. In addition, single-channel conductance is also significantly influenced by binding of these organic cations to a hydrophobic site located just to the cytoplasmic side of this selectivity filter.

We would like to thank Dr. Allan Lindsay for his help in the preparation of the purified Ca\(^{2+}\)-release channel and with elements of the computing.

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