Ion Selectivity of Porcine Skeletal Muscle Ca^{2+} Release Channels is Unaffected by the Arg^{615} to Cys^{615} Mutation

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ABSTRACT The Arg^{615} to Cys^{615} mutation of the sarcoplasmic reticulum (SR) Ca^{2+} release channel of malignant hyperthermia susceptible (MHS) pigs results in a decreased sensitivity of the channel to inhibitory Ca^{2+} concentrations. To investigate whether this mutation also affects the ion selectivity filter of the channel, the monovalent cation conductances and ion permeability ratios of single Ca^{2+} release channels incorporated into planar lipid bilayers were compared. Monovalent cation conductances in symmetrical solutions were: Li^+, 183 pS ± 3 (n = 21); Na^+, 474 pS ± 6 (n = 29); K^+, 771 pS ± 7 (n = 29); Rb^+, 502 pS ± 10 (n = 22); and Cs^+, 527 pS ± 5 (n = 16). The single-channel conductances of MHS and normal Ca^{2+} release channel were not significantly different for any of the monovalent cations tested. Permeability ratios measured under biionic conditions had the permeability sequence $Ca^{2+} \gg Li^+ > Na^+ > K^+ \geq Rb^+ > Cs^+$, with no significant difference noted between MHS and normal channels. This systematic examination of the conduction properties of the pig skeletal muscle Ca^{2+} release channel indicated a higher Ca^{2+} selectivity ($P_{Ca^{2+}}$:P_{K^+} approximately 15.5) than the sixfold Ca^{2+} selectivity previously reported for rabbit skeletal (Smith et al., 1988) or sheep cardiac muscle (Tinker et al., 1992) Ca^{2+} release channels. These results also indicate that although Ca^{2+} regulation of Ca^{2+} release channel activity is altered, the Arg^{615} to Cys^{615} mutation of the porcine Ca^{2+} release channel does not affect the conductance or ion selectivity properties of the channel.

INTRODUCTION

The Ca^{2+} release channel of striated muscle controls the efflux of Ca^{2+} from its intracellular storage site in the sarcoplasmic reticulum (SR) (Imagawa et al., 1987; Lai et al., 1988). The skeletal muscle Ca^{2+} release channel is composed of four identical 565 kDa subunits, which form a large conductance channel that is strongly cation-selective and permeable to both monovalent and divalent cations (Smith et al., 1988). The deduced amino acid sequence of the skeletal muscle Ca^{2+} release channel has been obtained, and the structure has been predicted to contain from 4 to 12 transmembrane regions in the carboxy-terminal region, with the remainder of the protein forming a large cytoplasmic structure (Takeshima et al., 1989; Zorzato et al., 1990). Although putative Ca^{2+} and ATP binding domains have been identified (Chen et al., 1992; Chen et al., 1993), the precise location of ligand binding, regulatory, and channel-forming domains of this channel have not yet been defined.

An arginine to cysteine mutation at amino acid residue 615 (in the proposed cytoplasmic region) in the skeletal muscle Ca^{2+} release channel is highly correlated with the inherited skeletal muscle disorder malignant hyperthermia (MH) of pigs and certain human families (Fujii et al., 1991; Hogan et al., 1992; Gillard et al., 1991; Otsu et al., 1991). Indeed, SR isolated from MH-susceptible (MHS) pig muscle demonstrates an increased rate of Ca^{2+} release and an altered [^{3}H]ryanodine binding activity (Louis et al., 1992; Mickelson et al., 1990). At the single-channel level, the MHS pig skeletal muscle Ca^{2+} release channel has been shown to be less sensitive to the inhibitory effects of Ca^{2+} than the normal Ca^{2+} release channel (Shomer et al., 1992; Fill et al., 1990).

Although it is clear that the regulation of the MHS porcine Ca^{2+} release channel activity is altered, the question of whether this mutation causes altered ion conduction properties remains controversial. Some authors have reported no difference in the single-channel conductance of MHS and normal Ca^{2+} release channels of pigs (Shomer et al., 1992; Valdivia et al., 1991) and humans (Fill et al., 1991). In contrast, Nelson (1992) reported than the Cs^+ conductance of MHS human channels was 46% greater than that of normal channels, and Fill et al. (1990) reported that the MHS porcine Ca^{2+} release channel Cs^+ conductance was 12% greater than that of normal channels. The latter authors also reported that in millimolar *trans* Ca^{2+} MHS Ca^{2+} release channel conductance was decreased more than normal channel conductance, which could indicate that MHS pig channels have an increased selectivity for Ca^{2+}. Such an alteration might have important implications for Ca^{2+} regulation in MHS muscle; if the permeability ratio $P_{Ca^{2+}}$:P_{K^+} is altered by the MHS mutation, this in turn might alter the rate of Ca^{2+} release and contribute to the abnormal Ca^{2+} regulation typical of MHS muscle. Given that the selectivity for Ca^{2+} versus K^+ ($P_{Ca^{2+}}$-P_{K^+}) has been reported as approximately 6 for the rabbit skeletal muscle Ca^{2+} release channel (Smith et al., 1986), it is likely that under physiological conditions, with lumenal SR concentrations of approximately 1 mM Ca^{2+} and 140 mM K^+ (Bers, 1991), potassium ions compete significantly with Ca^{2+} ions for conductance via the Ca^{2+} release channel. Altered channel conductance and selectivity properties resulting from single amino acid changes in the cystic fibrosis

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transmembrane regulator chloride channel results in cystic fibrosis in some human families (Dalemans et al., 1991; Tabcharani et al., 1993). Thus, a rigorous characterization of the conduction properties of the MHS and normal skeletal muscle Ca^{2+} release channel was needed to help clarify the nature of the defect in this channel and the role that this abnormal channel plays in MH.

To determine whether the MHS pig skeletal muscle Ca^{2+} release channel mutation results in altered conduction properties in addition to the previously described altered Ca^{2+} sensitivity (Shomer et al., 1992; Fill et al., 1990), the monovalent cation conductances for the Group IA monovalent cations (K^+, Na^+, Cs^+, Li^+, and Rb^+), and the permeability ratios for K^+ versus Ca^{2+}, as well as K^+ versus the Group IA monovalent cations were determined. Although detailed studies of the conduction properties of the cardiac muscle Ca^{2+} release channel (which is a different gene product than the skeletal muscle isoform) have been reported (Tinker et al., 1992), this is the first rigorous report of the conduction properties of the skeletal muscle isoform. This systematic examination of the conduction properties of the pig skeletal muscle Ca^{2+} release channel indicated a higher Ca^{2+} selectivity ($P_{Ca^{2+}}:P_{K^+}$ approximately 15.5) than the sixfold Ca^{2+} selectivity previously reported for rabbit skeletal (Smith et al., 1988) or sheep cardiac muscle (Tinker et al., 1992) Ca^{2+} release channels. No differences in cation conductance or selectivity between MHS and normal channels were observed, however, indicating that although Ca^{2+} regulation of Ca^{2+} release channel activity is altered, the Arg^{615} to Cys^{615} mutation of the porcine Ca^{2+} release channel does not affect the conduction properties of the channel.

MATERIALS AND METHODS

Materials

Pigs were obtained from the University of Minnesota Experimental farm, where they were part of a swine genetics herd. These pigs were bred from defined genetic strains known to be either homozygous normal or homozygous for the gene causing MH-susceptibility (Louis et al., 1992). These herds have been genotyped for the C1843 to T1843 mutation responsible for the Arg^{615} to Cys^{615} MHS Ca^{2+} release channel mutation (Otsu et al., 1991). Soybean phosphatidylcholine (PC), egg PC, and *n*-decane were obtained from Sigma Chemical Co. (St. Louis, MO); phospholipids for planar lipid bilayer studies were obtained from Avanti Polar Lipids (Alabaster, AL); 3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate (CHAPS) was obtained from Boehringer-Manheim (Indianapolis, IN).

Isolation of purified Ca^{2+} release channels

The CHAPS-solubilized, purified Ca^{2+} release channel was isolated by a modification of the sucrose gradient centrifugation technique developed by Lai et al. (1988). Briefly, SR membranes (1.3 mg/ml) were solubilized in 1 M NaCl, 25 mM PIPES (pH 7.1), 0.5% CHAPS, 0.25% PC, plus a protease inhibitor cocktail containing 2 mM dithiothreitol, 0.8 mM benzamidine, 0.1 mM PMSF, 0.6 μg/ml pepstatin, 1 μg/ml leupeptin, 1 μg/ml aprotinin for 1 h with gentle agitation. Insoluble material was removed by centrifugation at 100,000 × *g* for 30 min. The supernatant was applied to 5–20% linear sucrose gradients containing 1 M NaCl, 25 mM PIPES (pH 7.1), 0.5% CHAPS, 0.25% PC, plus the protease inhibitors, and the tubes were centrifuged at 40,000 × *g* for 16 h at 4°C. Fractions containing the purified Ca^{2+} release channel were pooled and concentrated with an Amicon centriprep 30 and stored at −70°C until use. Immediately before use, 20-μl aliquots were diluted by the addition of 40 μl of a solution of 0.5% PC, 1% CHAPS; this dilution was found to increase the number of successful channel incorporations.

Single-channel studies

Mueller-Rudin planar lipid bilayers were formed by painting a lipid mixture across a 250-micron aperture in a Delrin cup. The lipid mixture comprised bovine brain phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine dissolved in *n*-decane (50 mg/ml) in a 5:3:2 ratio by weight. Before the addition of the purified Ca^{2+} release channel, BSA (3 mg/ml) was added to the *cis* chamber to reduce the nonspecific binding of Ca^{2+} release channel protein to sites in the bilayer chamber; the BSA alone had no effect on bilayer properties (data not shown).

The single channel recording solution used to examine monovalent cations contained 210 mM *X*Cl (where $X = K^+$, Na^+, Cs^+), 20 mM MOPS, pH 7.4 or 200 mM *X*Cl (where $X = Li^+$ or Rb^+), 20 mM MOPS, 15 mM Tris, pH 7.4. Monovalent cation conductances were determined from current voltage plots in symmetric recording solutions. Permeability ratios of monovalent cations were determined from the reversal potential (E_r) of the Ca^{2+} release channel under biionic conditions such that the K^+ recording solution was in one chamber, and the recording solution for the cation being tested was in the other chamber. A solution-changing apparatus was used to rapidly exchange solutions. Because millimolar concentrations of calcium on the *cis* side of the Ca^{2+} release channel result in inactivation of the channel (Shomer et al., 1992; Fill et al., 1990), all measurements of permeability ratios of K^+ versus Ca^{2+} were made with 210 mM K^+, 20 mM MOPS (pH 7.4) in the *cis* chamber and 50 mM $CaCl_2$, 20 mM MOPS (pH 7.4) in the *trans* chamber. All experiments were performed at room temperature (22–23°C).

Data acquisition and analysis

Analog data from a custom-designed headstage and amplifier was filtered at 1500 Hz through an 8-pole Bessel filter and digitized at 2000–5000 Hz (TL1 DMA Interface, Axon Instruments) for storage on a Bernoulli T-90 disk drive (Iomega Devices). Single-channel data were collected using either a *step protocol* or a *pulsing protocol*. In the *step protocol*, the potential was held at 0 mV for 2–4 s in between steps of 400–2000 ms duration to the test potential. In the *pulsing protocol*, the potential was held at 0 mV, stepped to the test potential for 200–1000 ms, then stepped to a second test potential of the same duration and magnitude but opposite in sign to the first test potential (CLAMPEX program, PCLAMP Software, Axon Instruments).

Current voltage relationships were constructed from current amplitudes of channel open events measured with the program FETCHAN (PCLAMP software, Axon Instruments). Monovalent cation conductances were calculated from the slope of the linear regression through current-voltage relationships of Ca^{2+} release channels over the range −100 to +100 mV in symmetrical monovalent recording solutions. Permeability ratios were derived from current voltage curves under biionic conditions. Linear regressions of the points from +30 to +100 mV and from −30 to −100 mV were used to extrapolate the reversal potentials for each of the two ionic currents. The biionic equation (Eq. 1) was used to calculate the permeability ratio $P_A:P_B$ of two monovalent cations, and the Fatt-Ginsborg equation (Eq. 2) was used to calculate the permeability ratio $P_{Ca^{2+}}:P_{K^+}$, where $E_{reversal}$ is the mean reversal potential of the *cis* and *trans* current, R is the gas constant, T is the temperature in K, F is the Faraday's constant, and z is the valence.

$$E_{reversal} = \frac{RT}{Fz} \ln\left(\frac{P_A[A]_{trans}}{P_B[B]_{cis}}\right) \quad (1)$$

$$E_{reversal} = \frac{RT}{2F} \ln\left(\frac{4P_{Ca}[Ca]_{trans}}{P_K[K]_{cis}}\right) \quad (2)$$

Sample means were compared using the Student's t-test (or paired t-test, when indicated) to determine statistical significance. The Bootstrap test (an iterative form of the rank-sum test) was also used to determine statistically significant differences between the means of small samples of data ($n < 5$) (Efron and Tibshirani, 1986). Results are presented as the mean ± SE unless indicated.

RESULTS

Single-channel conductance of MHS and normal Ca^{2+} release channels

To determine whether MHS Ca^{2+} release channel conductance was different from that of the normal Ca^{2+} release channel, current voltage curves were derived in symmetric solutions of the group IA monovalent cations. Representative single-channel traces are shown in Fig. 1 *A*. Current voltage relationships for both MHS and normals Ca^{2+} release channels were linear over the voltage range −100 to +100 mV, indicating that these channels were nonrectifying (Fig. 1 *B*). MHS and normal Ca^{2+} release channel conductances were not different for any monovalent cation examined (Fig. 1 *B*, Table 1). The mean conductances (MHS and normal pooled) were 771 ± 7 nS (n = 29) for K^+, 527 ± 5 nS (n = 16) for Cs^+, 502 ± 10 nS (n = 22) for Rb^+, 474 ± 6 nS (n = 29) for Na^+, and 183 ± 3 nS (n = 21) for Li^+.

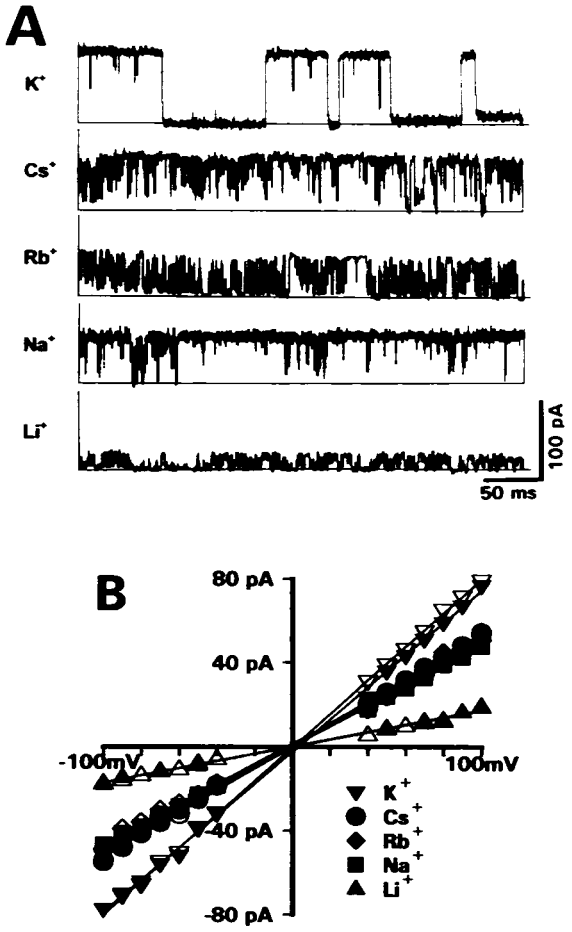

FIGURE 1 Conductance of purified MHS and normal Ca^{2+} release channels. (*A*) Representative single channel recordings of Ca^{2+} release channels in symmetrical monovalent solutions as described in Materials and Methods (200 mM XCl, 20 mM MOPS, 15 mM Tris, pH 7.4 for $X = Li^+$ or Rb^+, or symmetrical 215 mM XCl, 20 mM MOPS, pH 7.4 for $X = K^+$, Cs^+, or Na^+). Data was recorded using the *step protocol* at a holding potential of −70 mV for a duration of 400 ms. (*B*) Current-voltage curves of MHS (*filled symbols*) and normal (*open symbols*) channels recorded in symmetrical solutions as described above. Points represent the means ± SE of current amplitude determinations for 8–19 Ca^{2+} release channels; lines are the best-fit linear regressions through the data points.

Monovalent cation selectivity of MHS and normal Ca^{2+} release channels

To determine whether MHS Ca^{2+} release channel cation selectivity was different from that of the normal channels, current voltage relationships for MHS and normal channels were derived under biionic conditions, with 210 mM KCl, 20 mM MOPS (pH 7.4) in the *cis* chamber and 200 or 210 mM Cs^+, Rb^+, Na^+, or Li^+ in the *trans* chamber (Fig. 2). At potentials in the range −20 to +20 mV, the current voltage relationship was nonlinear. Furthermore, because the current amplitudes were small, especially for the lower-conductance cations such as Li^+, the low (<5) signal-to-noise ratio made accurate determination of the current amplitude difficult. For these reasons, linear regression analysis of the data from +30 to +100 mV and −30 to −100 mV (the range over which the current voltage relationship was linear) were used to derive the reversal potentials for the K^+, Cs^+, Rb^+, Na^+, and Li^+ currents. Permeability ratios determined with *trans* KCl solution and *cis* Cs^+, Rb^+, Na^+, or Li^+ solution were not distinguishable from the ratios determined in *cis* KCl and *trans* Cs^+, Rb^+, Na^+, or Li^+, so data from these two conditions were pooled.

MHS and normal Ca^{2+} release channel permeability ratios were not significantly different for any of the monovalent cations examined (Table 2). The mean permeability ratios $P_{K^+}:P_{X^+}$ were Li^+ 0.64 ± 0.04 (n = 6), Na^+ 0.76 ± 0.03 (n = 12), Rb^+ 1.10 ± 0.02 (n = 6), and Cs^+ 1.46 ± 0.07 (n = 16), yielding a selectivity sequence $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$.

TABLE 1 Monovalent cation conductance of MHS and normal Ca^{2+} release channels

Cation	Single channel conductance (pS)		
	Normal	MHS	Average
K^+	769 ± 9 (15)	773 ± 11 (14)	771 ± 7 (29)
Cs^+	523 ± 5 (8)	530 ± 7 (8)	527 ± 5 (16)
Rb^+	489 ± 15 (12)	517 ± 11 (10)	502 ± 10 (22)
Na^+	480 ± 12 (10)	471 ± 6 (19)	474 ± 6 (29)
Li^+	178 ± 4 (10)	186 ± 4 (11)	183 ± 3 (21)

Data are expressed as mean ± SE (n = number of channels analyzed) of MHS and normal channels recorded in symmetrical monovalent solutions as described in Materials and Methods. Recording solutions were 200 mM XCl, 20 mM MOPS, 15 mM Tris, pH 7.4 for $X = Li^+$ or Rb^+, or symmetrical 215 mM XCl, 20 mM MOPS, pH 7.4 for $X = K^+$, Cs^+, or Na^+.

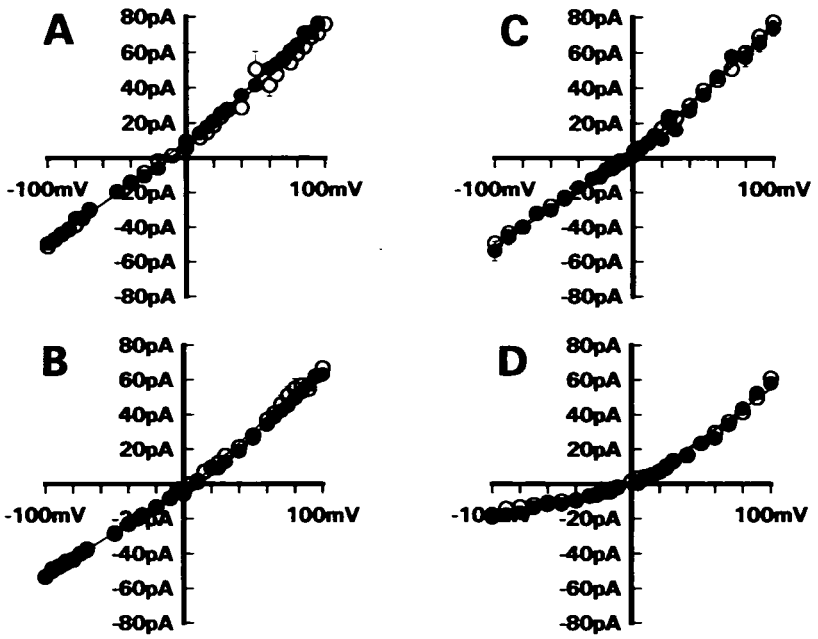

FIGURE 2 Monovalent cation selectivity of MHS and normal Ca^{2+} release channels. Current-voltage relationships of MHS (●) and normal (○) Ca^{2+} release channels in *cis* 215 mM KCl, 20 mM MOPS, pH 7.4 and *trans* (*A*) 215 mM CsCl, 20 mM MOPS, pH 7.4; (*B*) 215 mM NaCl, 20 mM MOPS, pH 7.4; (*C*) 200 mM RbCl, 20 mM MOPS, 15 mM Tris, pH 7.4; or (*D*) 200 mM LiCl, 20 mM MOPS, 15 mM Tris, pH 7.4. Points represent means ± SE for 3–7 MHS and normal Ca^{2+} release channels.

TABLE 2 Cation selectivity of MHS and normal Ca^{2+} release channels

	Cation permeability ratios		
Cation	MHS	Normal	Average
Cs^+	1.55 ± 0.10 (4)	1.39 ± .05 (4)	1.46 ± .07 (8)
Rb^+	1.07 ± 0.01 (2)	1.12 ± .04 (4)	1.10 ± .02 (6)
K^+	1	1	1
Na^+	0.73 ± .01 (7)	0.80 ± .02 (5)	0.76 ± .03 (12)
Li^+	0.67 ± .07 (3)	0.62 ± .06 (3)	0.64 ± .04 (6)
Ca^{2+}	18.5 ± 4.0 (4)	14.5 ± 3.2 (4)	15.5 ± 3.7 (8)

Permeability ratios were derived from current-voltage relationships of MHS and normal Ca^{2+} release channels measured in biionic conditions as described in Materials and Methods. Monovalent cation permeability ratios are expressed as the mean (P_{K^+}:P_{X^+}) values ± SE (n = number of channels analyzed) for MHS and normal Ca^{2+} release channels. The permeability ratio of Ca^{2+}:K^+ is expressed as the mean values ($P_{Ca^{2+}}$:P_{K^+}) ± SE.

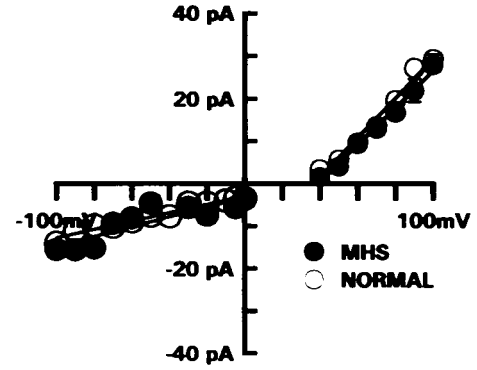

FIGURE 3 Ca^{2+} selectivity of MHS and normal Ca^{2+} release channels. Current voltage relationships of MHS (●) and normal (○) Ca^{2+} release channels in *cis* 215 mM KCl, 20 mM MOPS, pH 7.4, *trans* 50 mM $CaCl_2$, 20 mM MOPS, pH 7.4. Points represent means ± SE for 4 MHS and 4 normal Ca^{2+} release channels.

Divalent cation selectivity of MHS and normal Ca^{2+} release channels

To determine whether the divalent cation selectivity of the MHS Ca^{2+} release channel differed from that of the normal Ca^{2+} release channel, current voltage relationships for four MHS and four normal Ca^{2+} release channels were compared in *cis* 210 mM KCl, 20 mM MOPS (pH 7.4), and *trans* 50 mM $CaCl_2$, 20 mM MOPS, 15 mM Tris (pH 7.4). Potassium was chosen as the monovalent cation for three reasons: it had the largest conductance and so would provide the greatest signal-to-noise ratio; its conductance fell in the middle of the monovalent cation selectivity sequence; it is the predominant sarcoplasmic monovalent cation. Under these conditions, current voltage relationships were linear over the ranges −100 to −20 mV and +60 to +100 mV, but became markedly curvilinear near the reversal potential of approximately 30 mV (Fig. 3). Therefore, the linear regressions through the points from −100 to −20 mV and from +60 to +100 mV were used to derive the reversal potentials of the Ca^{2+} and K^+ currents, respectively. The permeability ratio $P_{Ca^{2+}}$:P_{K^+} was 18.5 ± 4.0 for the MHS Ca^{2+} release channels and 14.5 ± 3.7 for the normal Ca^{2+} release channels. These values were not significantly different by the Student's t-test or by the Bootstrap test.

DISCUSSION

Several previous studies have indicated that monovalent cation conductance and Ca^{2+} selectivity of the SR Ca^{2+} release channel are altered in MH (Fill et al., 1990; Nelson, 1992). Although such an alteration seems unlikely in light of the extra-transmembranous location of the Arg^{615} to Cys^{615} mutation, there are examples of naturally occurring and site-directed point mutations that result in altered channel conduction properties (Dalemans et al., 1991; Tabcharani et al.,

1993). Such mutations are all in transmembrane segments of the channel proteins; however, it has been hypothesized that charged luminal and cytoplasmic vestibules are important in ion conduction through the Ca^{2+} release channel (Tu and Fill, 1992). Thus, the loss of a positively charged arginine residue (Arg^{615}) in a cytoplasmic vestibule could result in altered ion conduction through the MHS channel. Furthermore, the Ca^{2+} release channel has been demonstrated to contain reactive thiols (Salama et al., 1992); the additional cysteine present in the MHS channel could have unpredictable effects on the conformation of the protein and on the channel conduction properties.

The principal finding of this study was that MHS and normal porcine Ca^{2+} release channel single-channel conductance and selectivity for monovalent and divalent cations did not differ, indicating that the increased Ca^{2+} release observed for MHS pig SR does not result from an increased Ca^{2+} selectivity. That the substitution of an uncharged cysteine for a positively charged arginine in the MHS Ca^{2+} release channel (Fujii et al., 1991) does not result in an altered conductance or selectivity suggests that the mutation is not in a portion of the protein that is proximal to either the channel pore or the conductance pathway, and that it does not result in a conformational change that alters the channel conductance properties. The lack of difference between the MHS and normal channel monovalent cation conductances reported here is consistent with previous reports for the MHS and normal purified pig Ca^{2+} release channel in symmetric Cs^+-containing solutions (Shomer et. al., 1992). Our result is in contrast, however, to that of Fill et al. (1990), who reported that the Cs^+ conductance of the MHS and normal pig Ca^{2+} release channels differed. The difference between our results and the previous work of Fill et al. (1990) might be explained by channel-to-channel variability, which was minimized in the present study because of the much larger number of channels examined. The difference between MHS and normal human Ca^{2+} release channel conductance observed by Nelson (1992) might result from the heterogeneous nature of MH in humans, because only a very small number of MHS human families have been shown to have the mutation homologous to the porcine Arg^{615} to Cys^{615} mutation (Gillard et al., 1991; Hogan et al, 1992).

The monovalent cation conductance properties of the purified pig skeletal muscle Ca^{2+} release channel reported here are comparable with those reported for the sheep cardiac muscle isoform of this channel (Tinker et al., 1992). The monovalent cation selectivity of the pig skeletal muscle channel (selectivity sequence $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$, corresponding to Eisenman sequence #11) is also similar to that reported for the sheep cardiac muscle Ca^{2+} release channel (Tinker et al., 1992), indicating that this property is highly conserved between species and isoforms. Our results are also largely compatible with the conductance properties observed for the rabbit (Smith et al., 1988) and human (Nelson, 1992) skeletal muscle Ca^{2+} release channels. However, the porcine skeletal muscle Ca^{2+} release channel exhibited selectivity against Cs^+ ($P_{Cs^+}:P_{K^+} = 0.68$) in contrast to the lack of selectivity among monovalent cations reported for the rabbit skeletal muscle channel (Smith et al., 1988). The sheep cardiac muscle channel has also been reported to discriminate significantly against Cs^+ ($P_{Cs^+}:P_{K^+} = 0.61$; Tinker et al., 1992). Furthermore, the porcine channel also exhibited a greater selectivity for Ca^{2+} ($P_{Ca^{2+}}:P_{K^+} = 15.5$) than was reported for the rabbit skeletal muscle Ca^{2+} release channel ($P_{Ca^{2+}}:P_{K^+} = 6$) (Smith et al., 1988) or for the sheep cardiac muscle Ca^{2+} release channel ($P_{Ca^{2+}}:P_{K^+} = 6$) (Tinker et al., 1992). Thus, selectivity might be partially determined by species- and isoform-specific factors.

The greater selectivity of the porcine channel for Ca^{2+} could be caused by a difference in the selectivity filter in the channel pore or possibly by a difference in the channel vestibule (the ion capture area). However, comparison of the amino acids in the 12 putative transmembrane portions of the rabbit skeletal and porcine skeletal muscle Ca^{2+} release channel sequence (Zorzato et al., 1990; Fujii et al., 1991) reveals complete conservation in all transmembrane regions except M3, which contains four amino acid differences ($A^{4279} - V^{4276}$, $A^{4281} - P^{4279}$, $V^{4286} - A^{4283}$, and $A^{4292} - L^{4289}$) with complete conservation of charge. Because the transmembrane regions of the rabbit and pig channels are virtually identical, whereas the selectivity and conductance properties of the channel are different, the conformation of the cytoplasmic and lumenal portions of the channel (the cytoplasmic and lumenal mouths) might contribute to channel selectivity. Other evidence that an extra-membranous region of the protein affects conductance and selectivity properties of the channel comes from the report of Tu and Fill (1992), who proposed that the lumenal mouth of the channel has a large concentration of negatively charged amino acid residues that concentrate cations, effectively conferring on the channel a large capture radius for Ca^{2+}.

The permeability ratio $P_{Ca^{2+}}:P_{K^+}$ indicates that K^+ ions compete with Ca^{2+} for permeation through the Ca^{2+} release channel. This suggests that under physiological conditions, K^+ ions would account for a significant fraction of the ion flux through the Ca^{2+} release channel, although there is likely no net K^+ current because the K^+ is equilibrated across the SR membrane via numerous K^+ channels. Assuming SR lumenal concentrations of 1 mM Ca^{2+} and 140 mM K^+ in vivo (Bers, 1991), $P_{Ca^{2+}}:P_{K^+} = 15.5$, negligible effects of other cations, negligible effects of the diffusion coefficients of Ca^{2+} and K^+, and single-ion occupancy of the channel, then the proportion of $Ca^{2+}:K^+$ flux would be: $([Ca^{2+}] \times P_{Ca^{2+}}:P_{K^+})/([Ca^{2+}] + [K^+])$, or 0.11. Because the ratio of K^+ to Ca^{2+} in the SR is large (140:1), small differences in selectivity could significantly affect the net Ca^{2+} flux through the SR Ca^{2+} release channel. However, our results indicate that the Arg^{615} to Cys^{615} mutation of the pig Ca^{2+} release channel does not result in an abnormality in the cation conductance or selectivity properties of this channel.

We conclude that the residue 615 mutation in the skeletal muscle Ca^{2+} release channel, which has been proposed to be located in the cytoplasmic region of the channel (Fujii et al., 1991), is likely not located proximal to the channel pore or

to the cytoplasmic vestibule/capture radius of the channel. Continued study of this naturally occurring mutation, supplemented with site-directed mutagenesis studies, will help to resolve issues of ion translocation through and regulation of the Ca^{2+} release channel.

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