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Characterization of the Calcium-release Channel/Ryanodine Receptor from Zebrafish Skeletal Muscle

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Abstract. Calcium (Ca^{2+})-mediated signaling is fueled by two sources for Ca^{2+}: Ca^{2+} can enter through Ca^{2+} channels located in the plasma membrane and can also be released from intracellular stores. In the present study the intracellular Ca^{2+} release channel/ryanodine receptor (RyR) from zebrafish skeletal muscle was characterized. Two RyR isoforms could be identified using immunoblotting and single-channel recordings. Biophysical properties as well as the regulation by modulators of RyR, ryanodine, ruthenium red and caffeine, were measured. Comparison with other RyRs showed that the zebrafish RyRs have features observed with all RyRs described to date and thus, can serve as a model system in future genetic and physiological studies. However, some differences in the biophysical properties were observed. The slope conductance for both isoforms was higher than that of the mammalian RyR type 1 (RyR1) measured with divalent ions. Also, inhibition by millimolar Ca^{2+} concentrations of the RyR isoform that is inhibited by high Ca^{2+} concentrations (teleost α RyR isoform) was attenuated when compared to mammalian RyRs. Due to the widespread expression of RyR these findings have important implications for the interpretation of the role of the RyR in Ca^{2+} signaling when comparing zebrafish with mammalian physiology, especially when analyzing mutations underlying physiological changes in zebrafish.

Key Words: Vertebrate — Sarcoplasmic reticulum — Caffeine — Intracellular calcium signaling — Ruthenium red — Teleost

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Introduction

In most cell types including muscle cells, neurons and epithelial cells intracellular calcium (Ca^{2+}) signaling is mediated, in part, by ryanodine receptors (Ca^{2+}-induced Ca^{2+} release channels, RyR). RyRs release Ca^{2+} ions from the sarcoplasmic/endoplasmic reticulum (SR/ER) into the cytosol and thereby convey the transduction of extracellular stimuli into intracellular Ca^{2+} signals or amplify and regulate the intracellular Ca^{2+} concentration. RyRs are large transmembrane proteins of 565 kDa that form tetrameric Ca^{2+} channels. They show sequence similarity with inositol 1,4,5-trisphosphate (IP_3)-gated Ca^{2+} channels of the SR/ER, but have distinct biophysical and pharmacological properties (Smith, Coronado & Meissner, 1986; Ehrlich & Watras, 1988; Supattapone et al., 1988; Mignery et al., 1989; Palade et al., 1989, Ehrlich et al., 1994). Ca^{2+} and adenine nucleotides have been shown to modulate RyR channel activity. Pharmacological agents that regulate RyR channel-open probability include caffeine, ryanodine, ruthenium red, protamine sulfate, heparin (Xu et al., 1998). There are three mammalian isoforms of the RyR which differ in their amino-acid sequence, cellular and tissue-specific distribution. Physiological differences among the three isoforms are based on the degree of activation by micromolar Ca^{2+} concentrations as well as phosphorylation-induced changes in channel properties (Witcher et al., 1991; Strand, Louis & Mickelson, 1993; Lamb, 2000). In nonmammalian vertebrates two distinct RyR isoforms termed α and β were identified (Sutko et al. 1991; Block, O'Brien & Meissner, 1996). These proteins are homologues of the mammalian type 1 and 3 RyR (Block et al., 1996; Ottini et al., 1996; Sonnleitner et al., 1998). Whereas in skeletal muscle of nonmammalian verte-

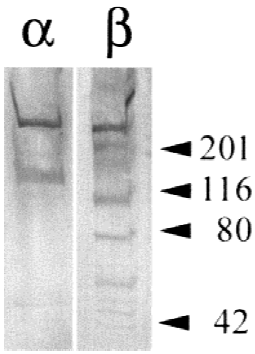

Fig. 1. Western blot showing the expression of α (left panel) and β (right panel) RyR isoforms by zebrafish skeletal muscle tissue. Membrane proteins of skeletal muscle SR vesicles (80 μg total protein per lane) were separated by SDS/PAGE in a 4–12% gradient gel. The primary antibodies directed against chicken RyR α and β each detected a band of ~500–600 kDa using peroxidase detection. The β isoform band has a slightly lower molecular weight than the α isoform. Numbers indicate the position and molecular weight of protein standards in kDa.

brates RyR α and β are expressed in approximately equal amounts (Ottini et al., 1996; this study), mammalian skeletal muscle predominantly expresses type 1 RyR and only small amounts of type 3 RyR (Takeshima et al., 1994, 1995). The co-expression of RyR α and β has been an important feature in a two-component model of Ca^{2+} release in skeletal muscle of nonmammalian vertebrates with two different forms of excitation-contraction coupling (O'Brien, Valdivia & Block, 1995). However, the biophysical and physiological properties of RyR function are strikingly similar when comparing different vertebrate and invertebrate species (Sorrentino, 1995; Quinn et al., 1998). In the present study the biophysical properties of RyRs from zebrafish skeletal muscle were characterized for the first time using single-channel recordings from RyRs incorporated in planar lipid bilayers. Data were compared to the predominant RyR isoform of mammalian skeletal muscle RyR1. Channel properties found in zebrafish skeletal muscle sarcoplasmic reticulum showed similarities to mammalian homologues indicating that physiological mechanisms for the regulation of intracellular Ca^{2+} signaling are preserved across vertebrate species. The results complement the current view of the role of RyR isoforms in nonmammalian vertebrate skeletal muscle and on the regulation of excitation-contraction coupling by RyRs. The present data provide a tool to assess the contribution of RyR function in cellular physiology of zebrafish, an emerging model animal for vertebrate ontogenesis, muscle and nerve cell developmental physiology, cellular development and differentiation.

Material and Methods

All experiments described in this study were carried out in accordance with the appropriate NIH, MBL and Yale University guidelines.

Immunoblotting of Zebrafish RyRs

Expression of RyRs α and β was monitored by immunoblotting using specific, affinity-purified rabbit polyclonal antisera directed against chicken RyR1 and RyR3 (Ottini et al., 1996; *see* Fig. 1; antibodies were kindly provided by Dr. V. Sorrentino). Immunoblotting was performed as previously described (Koulen et al., 2000) with aliquots of zebrafish skeletal muscle SR membrane vesicles that were also used in bilayer experiments, as described below. Immunoreactivity was visualized using the Vector VIP peroxidase substrate colorimetric detection system (Vector Laboratories, Burlingame, CA).

Bilayer Experiments

Zebrafish skeletal muscles from the dorsal body musculature, excluding muscles from the organs or the head, were dissected and homogenized. Vesicles from zebrafish skeletal muscle sarcoplasmic reticulum (SR) were isolated in the presence of protease inhibitors by differential centrifugation (Kim, Ohnishi & Ikemoto, 1983). The SR microsomes were incorporated into planar lipid bilayers (Ehrlich & Watras, 1988) containing phosphatidylethanolamine and phosphatidylserine (3:1 w/w; Avanti Polar Lipids, Alabaster, AL) dissolved in decane (40 mg lipid per ml) and RyR channel activity was monitored using Ba^{2+} ions as the current carrier (Koulen & Ehrlich, 2000). A KCl gradient with the higher KCl concentration on the side of vesicle incorporation (cis side) was used to facilitate fusion. The experiments were performed with a 250 mM HEPES-Tris solution, pH 7.35 on the cis and a 250 mM HEPES, 55 mM $Ba(OH)_2$ solution, pH 7.35 on the trans side of the bilayer. Pharmacological agents were applied to the cytosolic side of the channel (cis side). Ruthenium red, ryanodine and caffeine were purchased from Calbiochem (San Diego, CA) all other reagents were obtained from Sigma (St. Louis, MO). Data were recorded under voltage-clamp conditions, filtered at 1 kHz, digitized at 3 kHz and directly transferred to a computer. PClamp version 6.0.3 (Axon Instruments, Burlingame, CA) was used to acquire and analyze experiments. The distribution of open times was fitted to exponential curves using Marquardt least squares and statistical evaluation of the fit with χ^2-tests (pClamp version 6.0.3, Axon Instruments). The concentration of free Ca^{2+} was determined as described by Fabiato, 1988. Only channels that could be activated by addition of 1 μM Ca^{2+} to the cis side, as observed for the majority of experiments, were regarded as properly inserted into the artificial bilayer and were analyzed. Data shown in the present study were obtained from four or more independent trials for each experiment.

Results

Zebrafish Skeletal Muscle SR Contains RyRs α and β

SR vesicles prepared from zebrafish skeletal muscle were analyzed using standard Western blotting techniques and antisera specific for nonmammalian RyR isoforms (chicken RyR1 (α) and RyR3 (β); Ottini et al., 1996). Figure 1 shows that both antisera detect high-molecular weight bands of approximately 500-600 kDa.

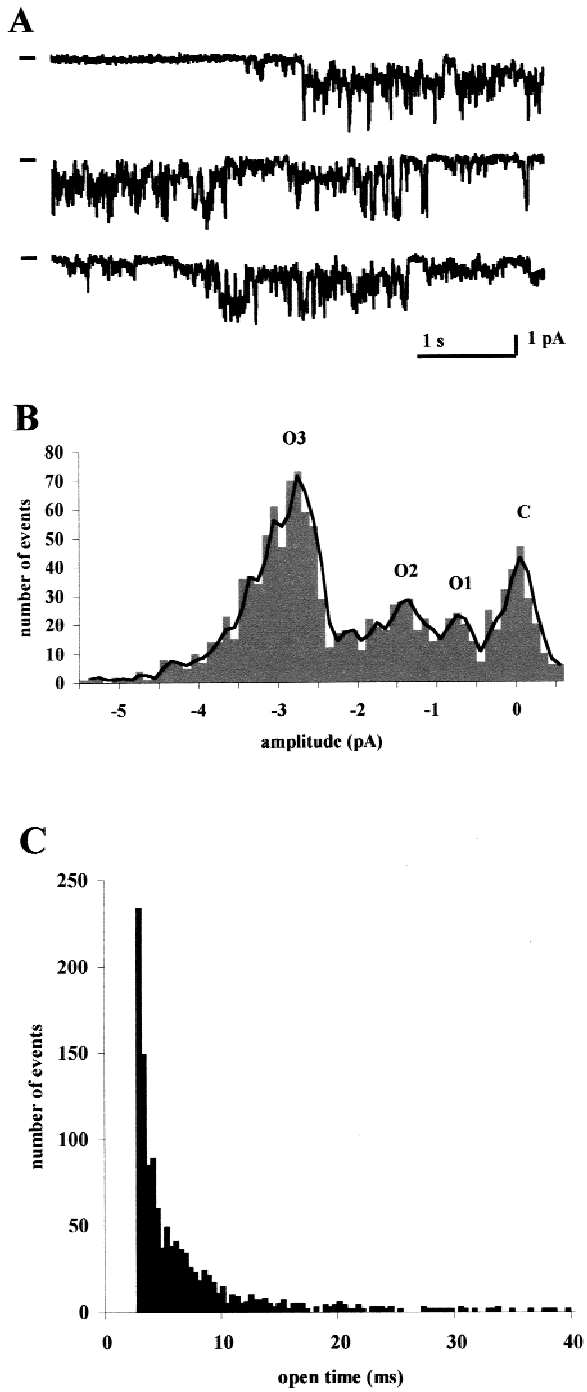

Fig. 2. Basal activity of zebrafish RyRs. (*A*) Traces from a single typical experiment monitoring activity of the higher conductance channel are shown. Full openings and distinct intermediate conducting states of the channel were observed. Channel openings are displayed as downward deflections. The zero-current baseline is indicated by bars on the left. (*B*) An amplitude histogram of a typical experiment analyzing channel activity of the higher conductance channel shows at least two subconductance states besides the fully open state and the closed state of the channel. The amplitude of the full channel opening was 2.7 ± 0.3 pA (*O3*), but the two sub-states with smaller amplitudes (*O1*, *O2*) could be observed frequently. The solid line connects the moving average of data points as a visual aid to better visualize subconductance states in the channel amplitude histogram. (*C*) The mean open time of the zebrafish RyR channel with higher conductance was 6.5 ± 2.4 msec. The distribution of open times was fit by an exponential curve using Marquardt least squares and statistical evaluation of the fit with χ^2-tests).

The β isoform immunoreactive band was of a slightly lower molecular weight than the α isoform immunoreactive band. This is in agreement with observations made for other nonmammalian vertebrate species (Airey et al., 1990; Olivares et al., 1991; Ottini et al., 1996). Taking into account that equal amounts of protein were loaded in each lane, the semi-quantitative immunoblot indicates that the two isoforms are expressed at similar concentrations.

The Pharmacological Profile of Zebrafish RyRs Is Identical to Mammalian RyRl

SR vesicles from zebrafish skeletal muscle were incorporated into planar lipid bilayers. Channels could be activated by 1 μM cytosolic free Ca^{2+} (Fig. 2*A*). Typically the active channel displayed at least two subconductance states besides the fully open state and the closed state of the channel. The current amplitude of the full channel

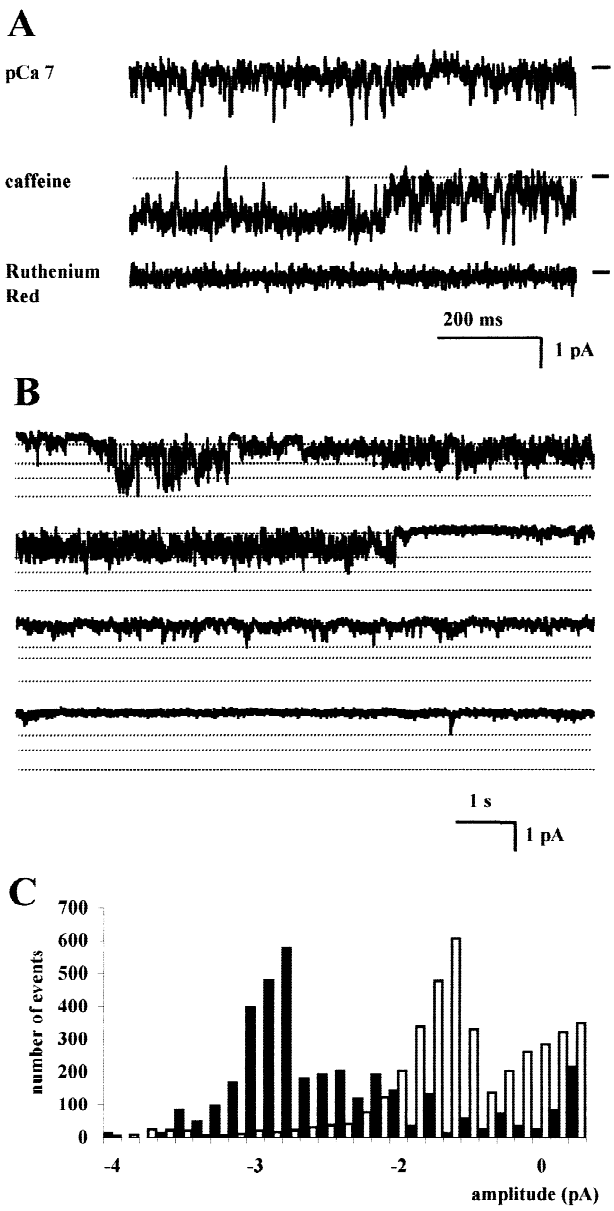

Fig. 3. Modulation of the zebrafish RyR by pharmacological agents known to affect mammalian RyRs. (*A*) Basal single channel activity was recorded in the presence of 100 nM free Ca^{2+} on the cytoplasmic side of the RyR (*pCa7;* top trace). The channel could be further activated with caffeine (10 mM; middle trace) and could be blocked with Ruthenium Red (3 μM; bottom trace). Traces from a single typical experiment monitoring activity of the channel with higher conductance are shown. Channel openings are displayed as downward deflections. The zero-current baseline is indicated by a bar on the right of each trace. In the middle trace a dotted line is added to indicate the baseline. (*B*) The zebrafish RyR was modulated by the addition of 100 nM ryanodine to the cytosolic side of the channel. The four consecutive traces show a continuous recording of a single channel starting 3 min after ryanodine addition. The zero-current baseline and three distinct conductance states are indicated by dotted lines for each trace (top line: closed state; bottom line: open state). As observed with mammalian RyRs, the current amplitude was reduced to a subconductance state (1 pA; upper middle line). Open probability declined with time. (*C*) Amplitude histogram of channel activity before (black) and after (white) addition of 100 nM ryanodine to the cytosolic side of the channel. Whereas in the control situation (black) full openings are predominant, ryanodine-treated channels open to substates and are more frequently closed (white). The analysis of one typical experiment measured at pCa 7 is shown.

opening was 2.7 ± 0.3 pA at a holding potential of 0 mV. Current amplitudes of 0.8 ± 0.2 pA and 1.3 ± 0.3 pA were measured for the two substates with smaller amplitudes (Fig. 2*B*). For the mammalian RyRs channel amplitudes of 2.5–3.9 pA have been observed (Bezprozvanny et al., 1991; Quinn et al., 1998; Koulen and Ehrlich, 2000). The mean open time of zebrafish RyRs at submaximal concentrations of 0.1 μM free Ca^{2+} at the cytosolic side was 7 ± 3 msec (Fig. 2*C*). These values again are similar to measurements obtained from mammalian RyRs using the same experimental conditions (Quinn et al., 1998). The effects of pharmacological agents known to affect mammalian RyRs were examined for zebrafish RyRs. The addition of 10 mM caffeine to the cytosolic side of submaximally activated RyR channels led to a pronounced increase in the channel-open probability (11-fold). Further addition of 2 μM and higher ruthenium red concentrations blocked channel activity completely and irreversibly (Fig. 3*A*). If channels were exposed to a concentration of 100 nM or higher of the alkaloid ryanodine the channel only opened to the 0.8 pA subconductance state within minutes after drug application (Fig. 3*B*). Continued exposure to ryanodine led to a decrease in open probability while the channel was locked in a subconductance state and did not open to a full conductance state (Fig. 3*B*). After treatment with ryanodine the number of RyR channel openings into a sub-conductance state increased while the occurrence of fully open channels decreased with time (Fig. 3*C*).

Two Types of Zebrafish RyR Channel Can Be Distinguished Based on Their Single-Channel Conductance

Further analysis of the biophysical properties of RyRs from zebrafish skeletal muscle revealed the presence of at least two physiologically distinct isoforms. When the current-voltage relationship of rabbit RyR1 was compared with that of zebrafish RyRs higher slope conductances were found for the zebrafish channels. Two distinct types of RyR channel could be distinguished by their slope conductance. In figure 4 experiments have been summarized and plotted together with results for the rabbit RyR1 for comparison (dashed line/circles: channels with lower conductance, $n = 4$; solid line/squares: channels with higher conductance, $n = 7$; dotted line/triangles: rabbit RyR1, $n = 6$). The conductances for each channel as determined by linear regression analysis clearly identify the isoforms biophysically (174 ± 7 pS for the channels with higher conductance, 149 ± 6 pS for the channels with lower conductance, and 104 ± 8 pS for the rabbit RyR1; correlation coefficients R^2 for the linear regression analysis were 0.97, 0.98 and 0.97, respectively). Note that the zebrafish RyR channel conductances are significantly larger when compared with a mammalian homologue under identical recording conditions. The value for rabbit RyR1 agrees with published data using the same recording conditions (Quinn et al., 1998) and with values obtained using similar conditions (Meissner 1986, 1994; Ehrlich et al., 1994). The two groups of zebrafish RyRs were identified based on the properties of single channel conductances of other nonmammalian vertebrate RyRs as α for the higher conductance channel and β for the lower conductance channel (Sutko et al. 1991; Block et al., 1994, 1996). Typical for the zebrafish RyRs, as seen for all RyRs described so far, was the lack of voltage activation.

Modulation of Zebrafish RyRs by Cytosolic Free Ca^{2+}

To further characterize the two distinct groups of RyR channels, the effect of different concentrations of free Ca^{2+} on the cytoplasmic side of the RyR was investigated. In all cases an increase in the free Ca^{2+} concentration from 10 nM, as found in the cytosol of unstimulated cells, to submicromolar concentrations led to an activation of RyR channels (Fig. 5*A, B,* top and middle traces). The single-channel open probability was 33 ± 11% at maximally activating Ca^{2+} concentrations (pCa 5.5 for the α and pCa 4.5 for the β isoform, Fig. 5*C*). Data from individual experiments were normalized before they were averaged, because activity levels vary among individual channels. This Ca^{2+}-induced increase in channel activity was very similar to the Ca^{2+}-dependence described for mammalian RyR (Smith et al., 1986; Bezprozvanny et al., 1991; rabbit RyR1 in Fig. 5*C*). A further increase in cytosolic free Ca^{2+}, however, showed two groups of channels distinguishable by their Ca^{2+}-dependent activity: One set of channels which had been shown to have a higher single-channel conductance (Fig. 4, the α isoform) showed inhibition by submillimolar and millimolar free cytosolic Ca^{2+} similar to mammalian RyR (Fig. 5*A, C*). The second group of RyR channels maintained maximal activity and was not inhibited even at millimolar Ca^{2+} concentration (Fig. 5*B, C*). This group was identical to the set of channels identified in Fig. 4 as the β isoform with a smaller single-channel conductance (149 pS) when compared with the α isoform (174 pS). Thus, a plateau of channel activity is reached for the β isoform. These data also show that the α isoform retains a residual activity at an elevated free cytosolic Ca^{2+} concentration of 1 mM. When compared to the rabbit RyR1 both the zebrafish α and β isoforms remain active at higher free cytosolic Ca^{2+} concentrations.

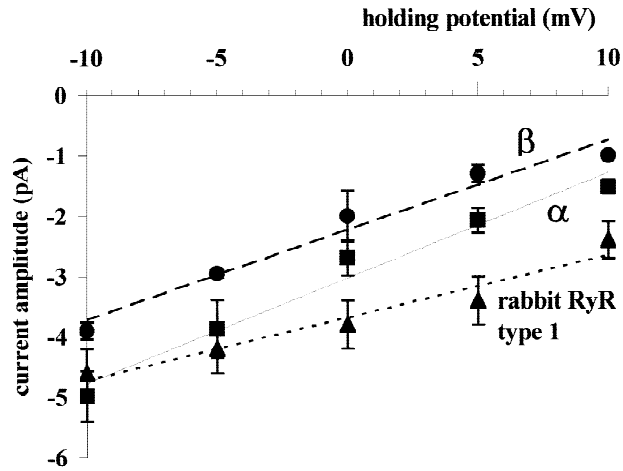

Fig. 4. Comparison of the current-voltage-relationship of rabbit RyR1 with those of zebrafish RyR isoforms. For each channel isoform the average amplitudes at given holding potentials were plotted (solid line/squares: channels with higher conductance, $n = 7$; dashed line/circles: channels with lower conductance, $n = 4$; dotted line/triangles: rabbit RyR1, $n = 6$). The conductance of each channel isoform was determined by linear regression analysis over the holding potential range of −10 to 10 mV. The slope conductances of the zebrafish RyR channels (174 ± 7 pS and 149 ± 6 pS respectively) were significantly higher than the one determined for their mammalian homologue (rabbit: 104 pS; correlation coefficients R^2 for the linear regression analysis were 0.97, 0.98 and 0.97, respectively). The two zebrafish RyR channels were identified based on their single-channel conductances as α (higher conductance) and β (lower conductance).

Discussion

In the present study zebrafish RyRs were shown to share a high degree of similarity in their biophysical charac-

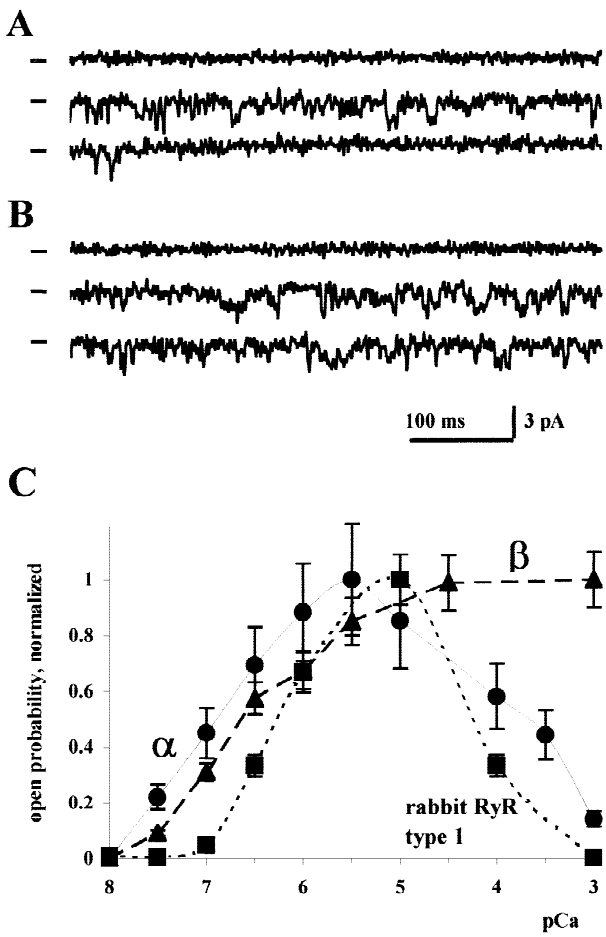

Fig. 5. Cytosolic free Ca^{2+} dependence of the zebrafish RyR isoforms. (*A*) Single-channel activity of the zebrafish RyR α isoform (higher conductance) was recorded in the presence of 10 nM (top trace), 10 μM (middle trace) and 1 mM (bottom trace) free Ca^{2+} on the cytoplasmic side of the RyR. Traces from a single typical experiment are shown. Channel openings are displayed as downward deflections. The zero-current baseline is indicated by a bar on the left of each trace. Inhibition of channel activity induced by micromolar free Ca^{2+} concentrations occurred when cytosolic free Ca^{2+} concentrations were raised to millimolar levels. (*B*) Recordings of single-channel activity of the zebrafish RyR β isoform (lower conductance) were monitored in the presence of 10 nM (top trace), 10 μM (middle trace) and 1 mM (bottom trace) free Ca^{2+} on the cytoplasmic side of the RyR. Traces from a single typical experiment are shown. Channel activity was induced by micromolar free Ca^{2+} concentrations but did not get inhibited when cytosolic free Ca^{2+} concentrations were raised to millimolar levels. Scale bars in *B* are for *A* and *B*. (*C*) The cytosolic free Ca^{2+} dependence of the normalized open probability of the zebrafish RyR α and β isoforms was compared with the rabbit RyR1. Individual points represent means ± standard error of the mean (SEM) for the zebrafish RyR β isoform (dashed line/triangles, $n = 4$), α isoform (solid line/circles, $n = 7$) and rabbit RyR1 (dotted line/squares, $n = 6$). If vertical error bars are not visible, the SEM of the open probability is smaller than the symbols. The zebrafish RyR α isoform and the rabbit RyR1 showed a bell-shaped activity dependence on cytosolic free Ca^{2+}. Inhibition required higher Ca^{2+} concentrations and at 1 mM free Ca^{2+} the zebrafish RyR α isoform was not fully inhibited. The zebrafish RyR β isoform was active at submicromolar cytosolic free Ca^{2+} concentrations, but was not inactivated by higher free Ca^{2+} concentrations.

teristics when compared with other nonmammalian vertebrate RyRs. Two types of functionally distinguishable RyRs were found, as in other teleosts (Block et al., 1994; O'Brien et al., 1995) and sauropsids (Sutko et al. 1991; O'Brien et al., 1995; Ottini et al., 1996). Ottini et al., 1996 could show that the homologues of the two isoforms in chicken, termed α and β isoform and identified based on biochemical and immunochemical properties (Airey et al., 1990, 1993; Olivares et al., 1991; Lai et al., 1992; O'Brien, Meissner & Block, 1993), are mammalian RyR1 and RyR3. In contrast to mammalian skeletal muscle, which expresses predominantly RyR1 and only small amounts of RyR3 (Takeshima et al. 1994, 1995), nonmammalian vertebrate skeletal muscle tissue was found to express both isoforms together at equal amounts (Fig. 1; Airey et al., 1990, 1993; Olivares et al., 1991; Lai et al., 1992; O'Brien et al., 1993). This finding has been interpreted both as a phylogenetic specialization using a 2-component model of Ca^{2+} release in skeletal muscle (O'Brien et al., 1995) and as a phylogenetic precursor form of the mammalian muscle types (Ottini et al., 1996).

Similarities of the Zebrafish RyRs with Mammalian Homologues

The RyR from zebrafish skeletal muscle sarcoplasmic reticulum shows similarities to mammalian homologues with regard to channel current amplitude, single-channel conductance, open states, dwell times and modulation by pharmacological agents (present paper; Bezprozvanny et al., 1991; Marks et al., 1989, Nakai et al., 1990; Kuwajima et al., 1992; Hakamata et al., 1992; Meissner 1986, 1994; Chen, Zhang & MacLennon, 1992; Chen & MacLennon, 1994). Zebrafish RyRs and their mammalian homologues are clearly distinct from other types of Ca^{2+} channel by their biophysical characteristics (Tkachuk, 2000), despite differences in the exact values for channel current amplitudes and single-channel conductance. As shown for mammalian homologues, the zebrafish RyR channels exhibited subconductance states, which are a typical functional property of channel ion gating (Meissner, 1986, 1994). These channel subconductance states are found more frequently when associated proteins, such as FKBP, dissociate from the RyR or during pharmacological modulation of the RyR by ryanodine (Fig. 3; Ehrlich et al., 1994; Kaftan, Marks & Ehrlich, 1996). The pharmacological profile of zebrafish RyRs also clearly relates them to mammalian RyR isoforms and distinguishes them from Ca^{2+} channels with similar biophysical properties, such as $InsP_3$ receptors (Ehrlich et al., 1994; Ghosh et al., 1988; Mignery et al., 1989; Palade et al., 1989). However, some hallmarks of zebrafish RyRs have to be noted as different from their mammalian counterparts in order to properly interpret their physiological function.

Specific Properties of Zebrafish RyRs Distinguishing Them from Mammalian RyRs

Significant, species-specific differences in the regulation by cytosolic Ca^{2+} and in single channel conductance were found for zebrafish RyRs when compared to mammalian RyRs. Zebrafish RyR channels with their higher slope conductance, a typical feature of nonmammalian vertebrate RyRs (Block et al., 1994; 1996; O'Brien et al., 1995), are capable of mediating a higher Ca^{2+} release from intracellular stores per channel and unit time than their mammalian counterparts (this study; Bezprozvanny et al., 1991). This could become important whenever channel kinetics and kinetics of Ca^{2+} transients are functionally relevant in cellular signaling. The 43% (β isoform) to 67% (α isoform) larger slope conductances of the zebrafish RyRs can, however, potentially create only small differences in the overall already fast and large release of Ca^{2+} from intracellular stores via RyRs, when compared to their mammalian homologues (Zahradnikova et al., 1999; Kim et al., 1983; Meissner, 1986).

The most dramatic difference of zebrafish RyRs to mammalian RyRs is the regulation of their activity by cytosolic free Ca^{2+} (this study; Bezprozvanny et al., 1991, Marks et al., 1989; Meissner, 1994). Two distinct sets of channels in zebrafish skeletal muscle were found. Both were activated by physiologically low Ca^{2+} concentrations, one was inactivated and the other one not affected by physiologically high Ca^{2+} concentrations. This matches results obtained from other nonmammalian vertebrate species (O'Brien et al., 1995; Oyamada et al., 1994). The predominant RyR isoform in mammalian skeletal muscle, RyR1, shows a similar activation by physiologically low Ca^{2+} concentrations but a distinct inactivation at high Ca^{2+} concentrations (this study; Bezprozvanny et al., 1991, Marks et al., 1989; Meissner, 1994). Mammalian RyR2 and RyR3 show a reduced sensitivity for high Ca^{2+} concentrations and remain more active under such conditions (Sonnleitner et al., 1998; Holmberg & Williams, 1990; Tu et al., 1994; Valdivia, 1998). The results of the present study show that zebrafish RyRs provide an excellent model system for mammalian physiology: The direct comparison of RyR function in nonmammalian and mammalian skeletal muscle has to be carefully evaluated given the different Ca^{2+} dependence of the channels involved. However, RyR3, albeit expressed at low levels, has been identified in mammalian skeletal muscle (Giannini et al., 1992, 1995) and it appears to be functionally important (Takeshima et al., 1995). The zebrafish RyR isoforms could become important model systems for RyR2 and RyR3 in tissues other than skeletal muscle, especially the central nervous system. The α isoform with its reduced sensitivity for physiologically high Ca^{2+} concentrations is very similar in its Ca^{2+} dependence of these mammalian RyRs. The β isoform with its lack of channel inactivation by Ca^{2+} could serve as a model to exaggerate functional properties of mammalian RyR2 and RyR3 behavior in cells.

Ca^{2+} signaling mediated by RyRs plays important roles in the development of cells and organs (Ferrari & Spitzer, 1999). The zebrafish serves in many disciplines as a model system for vertebrate development (Drummond, 2000; Paw & Zon, 2000; Malicki, 1999; Solnica-Krezel, 1999; Fetcho & Liu, 1998; Metscher & Ahlberg 1999). The results of the present study lay the foundation for a more detailed analysis of the contribution of RyRs to Ca^{2+}-mediated development of cells and tissues. In addition, the advanced genetic analysis and the technically well understood genetic manipulation of gene expression in zebrafish (Driever et al., 1994) will enable future research efforts using zebrafish as a genetic model for RyR regulation.

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