Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 1019–1022, February 1997
Physiology

Functional nonequality of the cardiac and skeletal ryanodine receptors

JUNICHI NAKAI*†, TATSUYA OGURA†, FELICIANO PROTASI‡, CLARA FRANZINI-ARMSTRONG‡, PAUL D. ALLEN§¶, AND KURT G. BEAM†‖

*Department of Information Physiology, National Institute of Physiological Science, Myodaiji-cho, Okazaki 444, Japan; †Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523; ‡Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA 19104-6058; §Department of Cardiology, Laboratory of Molecular Cardiology, Children's Hospital, Boston, MA 02115; and ¶Department of Anesthesiology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115

Contributed by Clara Franzini-Armstrong, November 25, 1996

ABSTRACT **Dihydropyridine receptors (DHPRs), which are voltage-gated Ca^{2+} channels, and ryanodine receptors (RyRs), which are intracellular Ca^{2+} release channels, are expressed in diverse cell types, including skeletal and cardiac muscle. In skeletal muscle, there appears to be reciprocal signaling between the skeletal isoforms of both the DHPR and the RyR (RyR-1), such that Ca^{2+} release activity of RyR-1 is controlled by the DHPR and Ca^{2+} channel activity of the DHPR is controlled by RyR-1. Dyspedic skeletal muscle cells, which do not express RyR-1, lack excitation–contraction coupling and have an ≈30-fold reduction in L-type Ca^{2+} current density. Here we have examined the ability of the predominant cardiac and brain RyR isoform, RyR-2, to substitute for RyR-1 in interacting with the skeletal DHPR. When RyR-2 is expressed in dyspedic muscle cells, it gives rise to spontaneous intracellular Ca^{2+} oscillations and supports Ca^{2+} entry-induced Ca^{2+} release. However, unlike RyR-1, the expressed RyR-2 does not increase the Ca^{2+} channel activity of the DHPR, nor is the gating of RyR-2 controlled by the skeletal DHPR. Thus, the ability to participate in skeletal-type reciprocal signaling appears to be a unique feature of RyR-1.**

Ca^{2+} regulates diverse cellular functions, and its movements are controlled by a variety of Ca^{2+} channels in the plasmalemma and endoplasmic reticulum. Amongst the important plasmalemmal Ca^{2+} channels are those sensitive to dihydropyridines, which are also known as dihydropyridine receptors (DHPRs) or L-type channels (1). Three gene families are known to exist for the L-type Ca^{2+} channels. One of these, the skeletal DHPR (2), is expressed at high levels only in skeletal muscle. The second, the cardiac DHPR (3), is expressed in heart, brain, and a number of other tissues. The third, class D (4), is expressed in neural and endocrine tissues. In the endoplasmic reticulum, ryanodine receptors (RyRs) are a prominent channel type (5), for which there are also three known gene families. RyR-1 (6, 7) is expressed at high levels in skeletal muscle and also in restricted regions of the brain. RyR-2 (8, 9) is the predominant isoform expressed in both the heart and brain (but also expressed in other tissues), and RyR-3 (10, 11) is expressed in diverse cell types.

Because Ca^{2+} is an important activator of RyRs, intracellular Ca^{2+} release can be initiated by Ca^{2+} entry through Ca^{2+} channels in the plasmalemma. Indeed, this kind of communication underlies excitation–contraction (EC) coupling in the heart: Ca^{2+} entering the cardiac DHPR activates RyR-2, thereby causing a large, additional release of Ca^{2+} from the sarcoplasmic reticulum (SR). A different kind of mechanism has evolved in skeletal muscle, where EC coupling results from an interaction between the skeletal DHPR and RyR-1, which does not require entry of extracellular Ca^{2+}. In dysgenic muscle cells, which lack the endogenous, skeletal DHPR, skeletal-type EC coupling is not restored by expression of cDNA encoding the cardiac DHPR, indicating that the DHPR isoform plays an important role in determining the nature of EC coupling (12). However, little direct information exists on whether the isoform of RyR is equally important in determining the type of EC coupling.

Recent work suggests that RyR-1 not only "receives" the EC coupling signal from the skeletal DHPR, but also transmits a retrograde signal back to the DHPR (13). Specifically, there is a large reduction in the density of L-type Ca^{2+} current in myotubes from dyspedic mice, which lack a functional copy of the RyR-1 gene (13, 14). Injection of these dyspedic myotubes with cDNA encoding RyR-1 restores both EC coupling and the density of L-type Ca^{2+} current, without changing the apparent number of DHPRs in the plasma membrane. Thus, RyR-1 appears to enhance the Ca^{2+} channel activity of the skeletal DHPR. A similar type of coupling has now also been described in cerebellar granule cells, where potentiation of L-type Ca^{2+} current is associated with activation of RyRs (15).

These results raise important questions about the molecular determinants that govern the occurrence and nature of coupling between L-type Ca^{2+} channels and RyRs. Dyspedic myotubes provide a useful system in which to address these questions. In the work described in this paper we have expressed RyR-2 cDNA in dyspedic myotubes to determine whether RyR-2 can substitute functionally for RyR-1. We found that RyR-2 can support Ca^{2+}-entry induced calcium release, but neither mediates skeletal-type EC coupling nor transmits the retrograde signal that enhances the Ca^{2+} channel activity of the skeletal DHPR.

EXPERIMENTAL PROCEDURES

Expression of cDNA. The coding sequence of the rabbit RyR-2 cDNA (8) was excised from pMAMCRR51 (16) by *Nhe*I and *Xho*I digestion and inserted into the expression plasmid pClneo (Promega). Dyspedic myotubes were cultured as described previously (13) and were subjected to nuclear injection (17) of cDNA 6–8 days after the initial plating of myoblasts. The injected myotubes were examined 1–3 days later.

Measurements of Ca^{2+} Transients and Membrane Currents. For experiments on intact myotubes, cells were loaded

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/97/941019-4$2.00/0
PNAS is available online at **http://www.pnas.org**.

Abbreviations: DHPR, dihydropyridine receptor; RyR, ryanodine receptor; EC, excitation–contraction coupling; SR, sarcoplasmic reticulum.

‖To whom reprint requests should be addressed. e-mail: KBeam@Vines.Colostate.edu.

with the acetoxymethyl ester of Fluo-3 (Molecular Probes; ref. 18). For spatially averaged measurements of fluorescence, a photomultiplier apparatus was used (19). For spatially resolved measurements, a charge-coupled device (CCD) camera was used to acquired images (30 frames per s) onto video tape. The images were subsequently digitized and analyzed with the National Institutes of Health IMAGE software.

The whole-cell patch clamp technique was used for the simultaneous measurement of Ca^{2+} currents and transients as described previously (19). Cells were stimulated with the prepulse protocol (20) to eliminate Na^+ and T-type Ca^{2+} currents. The patch pipette contained 140 mM Cs-aspartate, 5 mM $MgCl_2$, 10 mM Hepes (pH 7.4 with CsOH), 5 mM Na_2-ATP, and 0.1 mM or 10 mM Cs_2-EGTA. For the simultaneous measurement of Ca^{2+} transients, 0.2 mM pentapotassium-Fluo-3 was added to the pipette solution. The bath solution contained 145 mM tetraethylammonium$^+$, 165 mM Cl^-, 10 mM Hepes (pH 7.4 with CsOH), 0.003 mM tetrodotoxin, and either 10 mM Ca^{2+} (to support Ca^{2+} current) or 2 mM Ca^{2+}, 8 mM Mg^{2+}, 0.5 mM Cd^{2+}, and 0.3 mM La^{3+} (to block Ca^{2+} current). Temperature was 20–22°C.

Electron Microscopy. Dyspedic myotubes were grown on aclar coverslips coated with Matrigel (Collaborative Research) and injected with RyR-2 cDNA. Myotubes containing nuclear domains expressing RyR-2 (e.g., enlargement in Fig. 1*a*) were photographed for identification, after which the entire culture was fixed in glutaraldehyde (21), postfixed in 2% osmium-O_4 in 0.1 M cacodylate buffer, and enbloc-stained with saturated aqueous uranyl acetate at 60°C for 4 h. The cultures were embedded in Epon (Ted Pella) in two steps, using aclar to produce thin blocks of Epon with flat, unblemished surfaces, so that identified myotubes and specific nuclear domains could be easily visualized. A rectangle containing an identified myotube was cut out and carefully trimmed on one end with a cut transverse to the myotube and near the identified nuclear domain. Two thin layers of Epon were glued to the flat surface of the rectangle to stabilize for sectioning, leaving free the identified, trimmed end. Alternate thin and thick sections were cut across the myotube, stained with uranyl acetate followed by lead salts and viewed with a JEOL 1200 electron microscope.

RESULTS

Dyspedic myotubes, which expressed RyR-2 after cDNA injection, displayed regions of spontaneous contractions and within these regions had a rounded and vesiculated appearance (Fig. 1*a*). Spontaneous contractions are triggered by action potentials in normal myotubes (18). However, several observations indicate that the spontaneous contractions in RyR-2-injected myotubes are not triggered by action potentials but rather reflect spontaneous release of Ca^{2+} from the SR. First, unlike normal myotubes (17), RyR-2-injected dyspedic myotubes failed to contract in response to electrical field stimulation. Second, spontaneous Ca^{2+} transients in normal (Fig. 1*b*) and RyR-2-injected (Fig. 1*c*) myotubes differed markedly. In normal myotubes, the Ca^{2+} transient was nearly synchronous along the length of the myotube (Fig. 1*d*), consistent with triggering by an action potential. By contrast, RyR-2-injected myotubes typically displayed several separate foci of localized Ca^{2+} release, which sometimes culminated in a Ca^{2+} wave that slowly propagated along the myotube (e.g., Fig. 1*e*, propagation velocity of ≈0.5 μm/ms). These Ca^{2+} oscillations and waves were increased in frequency (Fig. 1*f*) and/or amplitude with 0.1 mM caffeine (n = 13/13), whereas ≥1 mM caffeine was required to cause a response in RyR-1-injected myotubes (13). The higher caffeine sensitivity of RyR-2-injected myotubes compared with RyR-1-injected myotubes may be a consequence of higher ambient Ca^{2+}, but it is also consistent with a previous observation that the purified cardiac RyR is more sensitive to caffeine than its skeletal counterpart (22).

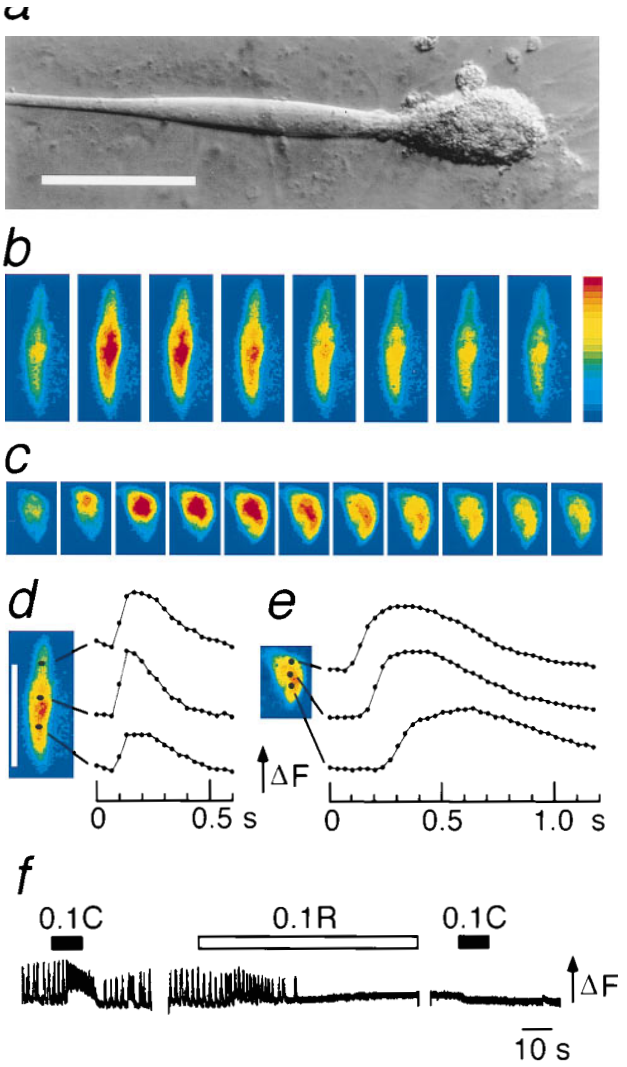

FIG. 1. Intracellular Ca^{2+} transients in intact dyspedic myotubes expressing RyR-2. (*a*) Photomicrograph showing the vesiculated appearance of a dyspedic myotube in the region injected with RyR-2 cDNA (enlargement on right). (Bar = 100 μm.) (*b* and *c*) Pseudocolor images, separated in time by 67 ms (*b*) or 100 ms (*c*), of spontaneous Ca^{2+} transients in a normal myotube and an RyR-2-injected dyspedic myotube, respectively. Color scale from blue to red indicates increasing Ca^{2+} concentration in arbitrary units of Fluo-3 fluorescence. (*d* and *e*) Fluorescence intensity as a function of time is plotted for the positions indicated on the adjacent images. (*d*, bar = 500 μm, and applies to *b*–*e*.) (*f*) Spatially averaged Ca^{2+} responses in a RyR-2-expressing dyspedic myotube; the bars indicate exposure to 0.1 mM caffeine (C) or 0.1 mM ryanodine (R).

The Ca^{2+} oscillations and caffeine responses in RyR-2-injected cells were attributable to the expressed RyR-2 because 0.1 mM ryanodine abolished the spontaneous Ca^{2+} activity, caused a slow increase in basal Ca^{2+}, and eliminated responsiveness to subsequent caffeine applications (Fig. 1*f*).

Expression of RyR-1 in dyspedic myotubes has been shown to restore skeletal-type EC coupling (i.e., coupling not dependent upon the entry of extracellular Ca^{2+}; ref. 13). As described above, field stimulation failed to elicit Ca^{2+} transients in RyR-2-injected myotubes. Depolarizing pulses applied with the whole-cell voltage-clamp technique also failed to elicit Ca^{2+} transients (Fig. 2*a*). Therefore, the gating of RyR-2 does not appear to be controllable by skeletal-type DHPRs in the plasmalemma. Furthermore, the Ca^{2+} channel activity of the skeletal DHPRs was not up-regulated by the presence of RyR-2, since slow, L-type Ca^{2+} currents remained small in

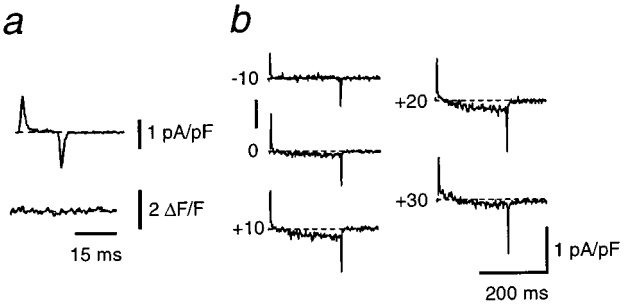

FIG. 2. Voltage-clamp analysis of a RyR-2-expressing dyspedic myotube. (*a*) Simultaneous measurement of membrane current (upper trace) and intracellular Ca^{2+} during a 15-ms depolarization to +20 mV. Depolarization failed to elicit an intracellular Ca^{2+} transient. (*b*) Expression of RyR-2 failed to restore expression of a large-amplitude slow Ca^{2+} current. Depolarizations of 200 ms to the indicated potentials. The patch pipette contained 10 mM EGTA and 200 μM Fluo-3 as Ca^{2+} buffers.

dyspedic cells expressing RyR-2 (Fig. 2*b*), with an average, peak current of only 0.17 ± 0.1 pA/pF (mean ± SD, $n = 10$). Therefore, unlike RyR-1 (13), RyR-2 lacks the ability to restore skeletal-type EC coupling or up-regulate skeletal L-type current in dyspedic myotubes.

As a further test of whether or not RyR-2 could support EC coupling, we coexpressed RyR-2 with CSk3 (23), a chimeric DHPR with a skeletal II–III loop in an otherwise entirely cardiac DHPR. We chose CSk3 because it supports skeletal-type EC coupling (23) and produces large, rapidly activating Ca^{2+} currents in both dysgenic myotubes (23) and nonmuscle cells (24). CSk3 also produced large currents in dyspedic myotubes (Fig. 3*a*): peak current was 13.5 ± 7.4 pA/pF ($n = 21$) in dyspedic myotubes injected only with CSk3 and 8.5 ± 5.8 pA/pF ($n = 8$) in dyspedic myotubes injected with both CSk3 and RyR-2. In intact dyspedic myotubes expressing CSk3 alone, field stimulation caused only small, rapidly decaying Ca^{2+} transients (Fig. 3*b*, top trace), whereas field stimulation evoked large, slowly decaying transients in dyspedic myotubes expressing both CSk3 and RyR-2 (Fig. 3*b*, middle trace). Thus, RyR-2 is capable of supporting Ca^{2+} release triggered by the CSk3 Ca^{2+} current. After block of Ca^{2+} current with Cd^{2+}/La^{3+}, field stimulation was no longer capable of evoking transients, although spontaneous transients still occurred (Fig. 3*b*, bottom trace). Since block of Ca^{2+} current does not abolish the skeletal-type EC coupling that occurs when CSk3 interacts with RyR-1 in dysgenic myotubes, we conclude that RyR-2 cannot mediate skeletal-type EC coupling. This observation is consistent with an earlier demonstration that fusion proteins containing DHPR II–III loops could activate RyR-1 but not RyR-2 (25).

In mammalian cardiac cells "feet" (the ultrastructural correlate of RyRs) are present in "corbular SR," which lacks junctional association with either the surface membrane or the transverse tubules, as well as in junctional SR associated with surface membrane or transverse tubules (26). If all or the great majority of RyR-2 in transfected dyspedic myotubes were located in corbular SR, this could account for the inability of RyR-2 to function in skeletal-type EC coupling. Therefore, electron micrographs were obtained for RyR-2-expressing myotubes. In these micrographs, a characteristic of the regions visually identified as expressing RyR-2 (cf. Fig. 1*a*) was a clearly higher activity of membrane assembly. Moreover, these regions displayed caveolae in the plasmalemma, evidence of formation of primitive transverse tubules (convoluted invaginations of the surface membrane) and junctional SR vesicles (as defined by a content of dense material, presumably calsequestrin). The majority of the SR vesicles were small and formed "peripheral couplings" with the surface membrane

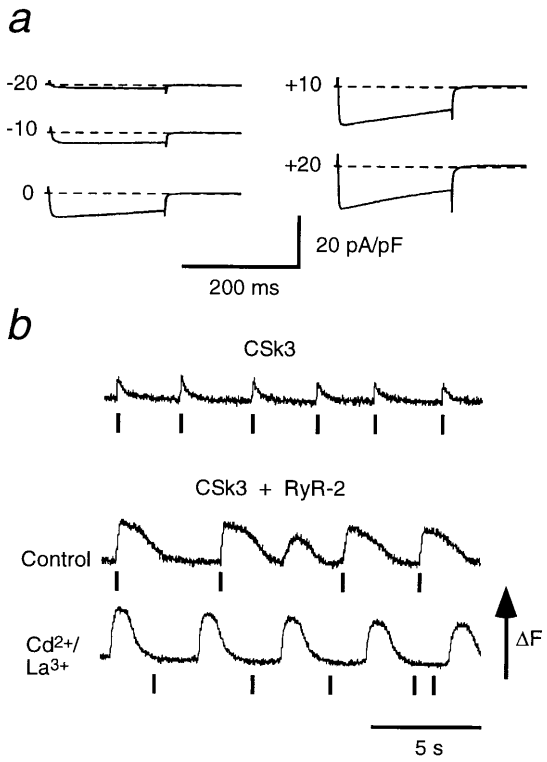

FIG. 3. Behavior of dyspedic cells expressing only CSk3 (a chimeric DHPR) or coexpressing CSk3 and RyR-2. (*a*) Presence of a large, rapidly activated calcium current in a voltage-clamped dyspedic myotube expressing CSk3. (*b*) Electrical stimulation (vertical bars) elicited only small, brief Ca^{2+} transients in an intact dyspedic myotube injected only with CSk3 cDNA (top trace). In an intact dyspedic myotube injected with both CSk3 and RyR-2 cDNA (middle trace), electrical stimulation elicited large, long-lasting Ca^{2+} transients (a spontaneous transient occurred between the second and third electrical stimuli). After addition of Cd^{2+} and La^{3+} to block Ca^{2+} current, electrical stimulation failed to evoke transients, although spontaneous transients still occurred (bottom trace, same cell as in middle trace). The cDNA for CSk3 (23) encodes a protein containing amino acids 1–787 and 923-2171 of the cardiac DHP receptor (3) joined by amino acids 666–792 of the skeletal DHP receptor (2).

(Fig. 4*a*) or "dyadic couplings" with membrane invaginations (Fig. 4*b*). Thus, there appeared to be little if any corbular SR. The junctions between the SR and plasmalemma in dyspedic muscle lack visible feet and have a narrower gap than junctions in normal muscle (27). Hence, we defined normal junctions as those having a visible pair of densities (feet) in the junctional gap and a sufficient gap width to accommodate feet. Of 66 junctions found in the four RyR-2-expressing myotubes examined, 23 were found to be normal, 27 were probably dyspedic (Fig. 4*c*), and the remaining 16 were equivocal (a single foot may have been present). The presence of normal junctions, together with the virtual absence of obvious, corbular SR, suggest that all the expressed RyR-2 is localized to junctions between the SR and plasmalemma.

DISCUSSION

Previous work (12) has shown that important differences between skeletal and cardiac muscle reside in the isoforms of the DHPR expressed in each tissue. The cardiac DHPR is well adapted for Ca^{2+} entry-dependent EC coupling because it produces a large, rapidly activating Ca^{2+} current. However, this is a maladaptive feature for skeletal muscle where large Ca^{2+} currents would tend to cause depletion of Ca^{2+} from the transverse tubules (28). The skeletal DHPR is adapted to produce a small, slowly activating Ca^{2+} current and trigger SR

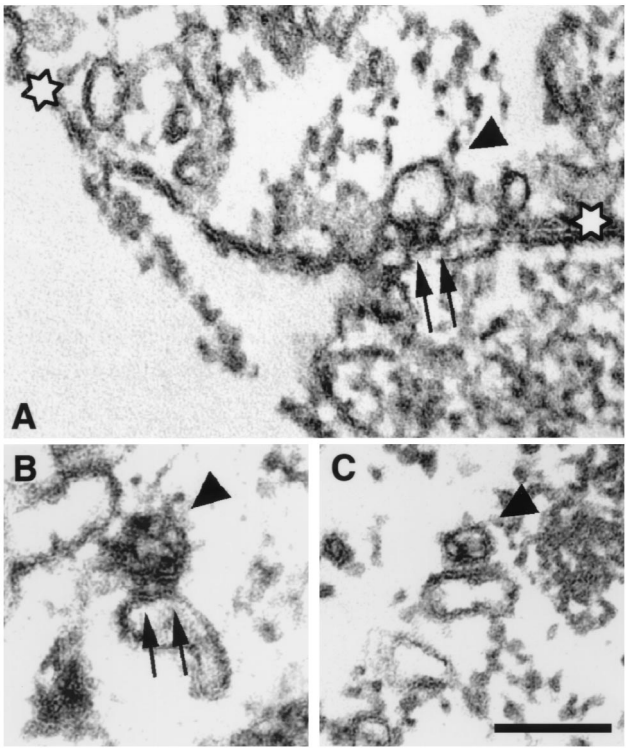

FIG. 4. Electron micrographs illustrating membrane junctions in RyR-2-expressing dyspedic myotubes. (*a*) A peripheral coupling between the SR (arrowhead) and plasmalemma (asterisks). A pair of feet (double arrows) spans the junctional gap. Extracellular debris is present in proximity of the cell (lower half of photograph). (*b*) A coupling which contains feet between the SR and a primitive transverse tubule. In addition to dyadic couplings (as shown here), there were also occasional triadic couplings (data not shown). The width of the junctional gaps containing feet was 13.2 ± 1.8 nm; 51 measurements). (*c*) A junction lacking feet between an SR vesicle and a plasmalemmal invagination in an RyR-2-injected myotube. Note that the junctional gap is smaller (9.0 ± 1.7 nm; 62 measurements) than in junctions with visible feet. Calibration = 0.1 μm (applies to *a*–*c*).

Ca^{2+} release by a mechanism that does not require Ca^{2+} entry. Here we have shown that RyR-1 and RyR-2 play an equally critical role in the mechanistic nature of EC coupling. RyR-2 lacks two essential attributes of RyR-1: (*i*) the ability to mediate skeletal-type EC coupling, and (*ii*) the ability to enhance Ca^{2+} channel activity of the skeletal DHPR. RyR-2 appears to be more susceptible to Ca^{2+}-induced Ca^{2+} release than RyR-1, since expression of RyR-2 in myotubes leads to repetitive Ca^{2+} releases not coupled to membrane excitation. An increased susceptibility of RyR-2 to Ca^{2+}-induced Ca^{2+} release is probably a useful adaptation for cardiac-type EC coupling. Nevertheless, healthy cardiac myocytes presumably have mechanisms that prevent uncoupled, repetitive Ca^{2+} releases.

RyR-1, RyR-2, and RyR-3 are found in many different tissues, including the brain, where all three are present (29, 30). The relative expression of the RyR isoforms varies dramatically between tissues and between regions of a given tissue, but the biological significance of this differential expression is unknown. The striking functional differences we have found between RyR-1 and RyR-2 are consistent with the idea that distinct neuronal functions are carried out by each of the RyR isoforms. It is intriguing to speculate as to whether some of these neuronal functions may make use of RyR attributes that are critical to muscle (e.g., reciprocal signaling with plasmalemmal calcium channels).

We thank N. Sekiguchi, R. Morris, and S. Mukherjee for technical help. This research was supported by a grant from the Japan Ministry of Education, Science, Culture and Sports to J.N., grants from Muscular Dystrophy Association and the Brigham and Women's Hospital Anesthesia Foundation (P.D.A.), and National Institutes of Health Grants HL48093 (C.F.-A.) and NS24444 (K.G.B.). T.O. was supported by National Institutes of Health Grants DC00244 and DC00766 (to S. C. Kinnamon).

1. Hofmann, F., Biel, M. & Flockerzi, V. (1994) *Annu. Rev. Neurosci.* **17,** 399–418.
2. Tanabe, T., Takeshima, H., Mikami. A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T. & Numa, S. (1987) *Nature (London)* **328,** 313–318.
3. Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, H., Narumiya, S. & Numa, S. (1989) *Nature (London)* **340,** 230–233.
4. Williams, M. E., Feldman, D. H., McCue, A. F., Brenner, R., Velicelebi, G., Ellis, S. B. & Harpold, M. M. (1992) *Neuron* **8,** 71–84.
5. McPherson, P. S. & Campbell, K. P. (1993) *J. Biol. Chem.* **268,** 13765–13768.
6. Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T. & Numa, S. (1989) *Nature (London)* **339,** 439–445.
7. Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N. M., Lai, F. A., Meissner, G. & MacLennan D. H. (1990) *J. Biol. Chem.* **265,** 2244–2256.
8. Nakai, J., Imagawa, T., Hakamata, Y., Shigekawa, M., Takeshima, H. & Numa, S. (1990) *FEBS Lett.* **271,** 169–177.
9. Otsu, K., Willard, H. F., Khanna, V. K., Zorzato, F., Green, N. M. & MacLennan, D. H. (1990) *J. Biol. Chem.* **265,** 13472–13483.
10. Hakamata, Y., Nakai, J., Takeshima, H. & Imoto, K. (1992) *FEBS Lett.* **312,** 229–235.
11. Giannini, G., Clementi, E., Ceci, R., Marziali, G. & Sorrentino, V. (1992) *Science* **257,** 91–94.
12. Tanabe, T., Mikami, A., Numa, S. & Beam, K. G. (1990) *Nature (London)* **344,** 451–453.
13. Nakai, J., Dirksen, R. T., Nguyen, H. T., Pessah, I. N., Beam, K. G. & Allen P. D. (1996) *Nature (London)* **380,** 72–75.
14. Takeshima, H., Iino, M., Takekura, H., Nishi, M., Kuno, J., Minowa, O., Takano, H. & Noda, T. (1994) *Nature (London)* **369,** 556–559.
15. Chavis, P., Fagni, L., Lansman, J. B. & Bockaert, J. (1996) *Nature (London)* **382,** 719–722.
16. Imagawa, T., Nakai, J., Takeshima, H., Nakasaki, Y. & Shigekawa, M. (1992) *J. Biochem.* **112,** 508–513.
17. Tanabe, T., Beam, K. G., Powell, J. A. & Numa, S. (1988) *Nature (London)* **336,** 134–139.
18. García, J. & Beam, K. G. (1994) *J. Gen. Physiol.* **103,** 107–123.
19. García, J., Tanabe, T. & Beam, K. G. (1994) *J. Gen. Physiol.* **103,** 125–147.
20. Adams, B. A., Tanabe, T., Mikami, A., Numa, S. & Beam, K. G. (1990) *Nature (London)* **346,** 569–572.
21. Takekura, H., Bennett, L., Tanabe, T., Beam, K. G. & Franzini-Armstrong, C. (1994) *Biophys. J.* **67,** 793–803.
22. Hymel, L., Schindler, H., Inui, M. & Fleisher, S. (1988) *Biochem. Biophys. Res. Commun.* **152,** 308–314.
23. Tanabe, T., Beam, K. G., Adams, B. A., Niidome, T. & Numa, S. (1990) *Nature (London)* **346,** 567–569.
24. Takekura, H., Takeshima, H., Nishimura, S., Takahashi, M., Tanabe, T., Flockerzi, V., Hofmann, F. & Franzini-Armstrong, C. F. (1995) *J. Muscle Res. Cell Motil.* **16,** 465–480.
25. Lu, X., Xu, L. & Meissner, G. (1994) *J. Biol. Chem.* **269,** 6511–6516.
26. Dolber, P. C. & Sommer, J. R. (1984) *J. Ultrastruct. Res.* **87,** 190–196.
27. Takekura, H., Nishi, M., Noda, T., Takeshima, H. & Franzini-Armstrong, C. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3381–3385.
28. Almers, W., Fink, R. & Palade. P. T. (1981) *J. Physiol. (London)* **312,** 177–207.
29. Furuichi, T., Furutama, D., Hakamata, Y., Nakai, J., Takeshima, H. & Mikoshiba, K. (1994) *J. Neurosci.* **14,** 4794–4805.
30. Giannini, G., Conti, A., Mammarella, S., Scorbogna, M. & Sorrentino, V. (1995) *J. Cell Biol.* **128,** 893–904.