Anti–Ryanodine Receptor Antibody Binding Sites in Vascular and Endocardial Endothelium

Ryan E. Lesh, Andrew R. Marks, Avril V. Somlyo, Sidney Fleischer, and Andrew P. Somlyo

The ryanodine receptor (RyR) functions as the calcium release channel of the sarcoplasmic reticulum activated by electromechanical coupling in skeletal and cardiac muscles. In smooth muscle, inositol trisphosphate releases calcium from internal stores during pharmacomechanical coupling, but these cells also contain ryanodine-sensitive calcium stores. In this study, we establish the presence of anti-RyR antibody binding sites in vascular and endocardial endothelium. Both types of endothelia also contain messenger RNA, which hybridizes to a cardiac RyR isoform cDNA probe. Western blots of endothelial cell homogenates demonstrate the presence of a single, high molecular weight band of protein that corresponds to the cardiac RyR isoform. Confocal micrographs of endothelial cells labeled with a specific anti-RyR antibody reveal an intense fluorescent signal surrounding the nucleus and distributed in a nonhomogeneous pattern throughout the cytoplasm. This pattern of fluorescence is consistent with the electron microscopic distribution of the endoplasmic reticulum. The pattern of immunofluorescence seen with the anti-RyR antibody is distinctly different from that seen with the mitochondrial fluorescent rhodamine 123. Our findings suggest that the RyR plays a role in endothelial signaling. (Circulation Research 1993;72:481–488)

KEY WORDS • ryanodine receptors • endothelium • confocal microscopy • immunofluorescence

Calcium plays an important role in endothelial cell signaling as a messenger for the release of endothelial factors regulating vascular smooth1,2 and cardiac3 muscle. In endothelial cells, as in many other eukaryotic systems,4 the release of intracellularly stored Ca2+, in addition to trans–plasma membrane fluxes, contributes to agonist-induced increases in cytoplasmic [Ca2+]i.5 Furthermore, endothelial cells, like other nonmuscle cells, contain a labyrinth of tubules and cisternae of endoplasmic reticulum (ER) that is similar to the sarcoplasmic reticulum of smooth muscle5 and can accumulate divalent cations.10 The Ca2+ content of this compartment can also be depleted by inhibitors (e.g., thapsigargin, a specific inhibitor of intracellular calcium pumps; see References 11 and 12) of the ER Ca2+ pump.5

The ryanodine receptor (RyR[s]) is the channel through which Ca2+ is released during normal excitation–contraction coupling (electromechanical coupling) in skeletal and in cardiac muscle.13 In smooth muscle (reviewed in References 14 and 15) and in nonmuscle cells,10 the inositol 1,4,5-trisphosphate receptor is the major functional Ca2+-release channel involved in pharmacomechanical coupling.14,15 but these cells may also contain RyRs. We now show the presence of RyRs in the endothelium of both the vasculature and endocardium, suggesting that these channels also play a role in endothelial signal transduction.

Materials and Methods

Tissue Preparation

Adult male guinea pigs (approximately 350 g, obtained from Hilltop Laboratory Animals, Scottdale, Pa.) were killed with an overdose of halothane anesthesia and then exsanguinated, following a protocol approved by the University of Virginia Animal Experimentation Committee, in accordance with policies outlined in the Public Health Service policy on humane care and use of laboratory animals. Blood vessels were dissected free and placed in HEPES-buffered normal Krebs’ solution at 37°C. The connective tissue was gently removed before fixation. Hearts were removed from the animals while still beating and transferred to a solution of bicarbonate-buffered Krebs’ solution at 32°C gassed with 95% O2–5% CO2, pH 7.3, and the connective tissue was removed using microdissection scissors and razor knives under a dissecting microscope, with care taken not to damage the endothelium. Strips of thoracic aorta, papillary muscle, and trabecular muscle were pinned to small dental wax strips at lengths approximating in vivo lengths and fixed overnight at 4°C in freshly prepared 3% paraformaldehyde in 10 mM phosphate-
buffered saline (PBS), pH 7.4. The fixed tissues were cut into 3-mm×8-mm pieces suitable for rapid freezing. Tissues were cryoprotected in 5% sucrose-PBS (wt/vol), pH 7.4, for 60 minutes at 4°C. Subsequently, they were placed in 15% sucrose-PBS, pH 7.4, at 4°C for an additional 45–60 minutes, then rapidly frozen in liquid N2-subcooled Freon-22,18 and kept in Freon-22 frozen with liquid N2 until cryosectioning.

Cryosectioning

Cryosectioning was performed on a Histostat cryomicrotome (model 855, American Optical). Tissues were allowed to warm to −20°C in the Histostat’s cryochamber and affixed to a cryosectioning chuck, with Tissue-Tek O.C.T. compound (Miles Inc., Elkhart, Ind.), in either a longitudinal or transverse orientation. Approximately 8–12-μm-thick sections were cut and collected on room-temperature gelatin-coated glass microscope slides. Sections were kept at −20°C in the cryochamber until they were transferred to storage at −80°C. Immunolabeling experiments were performed only on sections that were stored less than 2 days.

Indirect Immunolabeling

Paraformaldehyde-fixed tissue sections were prepared for confocal microscopy by immunolabeling with an affinity-purified, polyclonal rabbit anti-RyR primary antibody (anti-RyR 8 or anti-RyR 5). An affinity-purified, species-specific, tetramethylrhodamine isothiocyanate (TRITC)—conjugated F(ab′)2 fragment (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) was used for indirect labeling. Tissue sections, mounted on gelatin-coated glass microscope slides, were removed from storage at −80°C and allowed to warm to room temperature. Each section was preincubated in a solution of PBS with 3% bovine serum albumin (BSA), pH 7.4, for 10 minutes. This solution was aspirated from the slide, and 100 μl primary anti-RyR 8 or anti-RyR 5, diluted 1:50 (vol/vol) in 3% BSA-PBS solution, was placed on the sections for a 2-hour incubation, after which the antibody was aspirated from the slide and replaced with 3% BSA-PBS wash solution (250 μl) for 5 minutes. The wash solution was aspirated, and the wash procedure was repeated a total of four times. The tissue sections were then blocked for 5 minutes with a 5% solution (vol/vol) of preimmune donkey serum diluted in 3% BSA-PBS to minimize nonspecific binding of the secondary antibody. This was aspirated and replaced with the TRITC-labeled F(ab′)2 fragment at a final concentration of 10 μg/ml of 3% BSA-PBS solution. Tissue samples were incubated in the secondary F(ab′)2 fragment for 1 hour, washed as before with four washes of 3% BSA-PBS, and mounted with buffered glycerol containing antifade.

Rhodamine 123 Labeling

To image mitochondria, fresh specimens of guinea pig cardiac atrial trabeculae and aortas were labeled with rhodamine 123, essentially as described by Johnson et al.19 The aorta was cut into small strips (~3 mm×50 μm) with a diamond dissecting knife and incubated (without fixation) for 30 minutes at 37°C in 10 μg/ml rhodamine 123 in either HEPES-buffered Krebs’ (aortic tissue) or bicarbonate-buffered Krebs’ solution gassed with 95% O2–5% CO2 (cardiac tissue). The tissue was then washed in two changes of buffer and mounted on a glass microscope slide with a coverslip for imaging in the confocal microscope as described below. This fluorophore emitted bright green fluorescence when excited with blue light (488 nm).

cDNA Probes

The cardiac RyR cDNA probe used for Northern blot analysis was cloned from rabbit heart total RNA essentially as previously described.20 This cDNA clone, HCRCl, corresponds to nucleotides 5,027–5,647 of the cDNA encoding the rabbit cardiac RyR.21 Probes used for Northern blot analyses were uniformly labeled with random primers using Klenow reagent and [α-32P]dCTP to a specific activity of >108 cpm/μg.

RNA Preparation and Northern Blot Analysis

RNA was prepared from rabbit heart, freshly isolated human umbilical vein endothelium, and cultured bovine aortic endothelium (fourth passage) using standard guanidium-thiocyanate lysis buffer and centrifugation over a cesium chloride gradient as previously described.22 RNA was quantitated by spectrophotometry at 260 nm, and the ratio of absorbance at 260 to that at 280 nm was greater than 1.8 for all the samples. Total cellular RNA was size-fractionated on formaldehydeagarose gels run at 30 mA overnight to provide resolution of high molecular weight mRNAs. RNA transfer onto nitrocellulose filters was conducted overnight using 10× standard saline citrate (SSC) (1× SSC contains 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) transfer buffer. Filters were then baked at 80°C in a vacuum oven for 2 hours and prehybridized overnight in a buffer containing 1× Denhardt’s solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% BSA), 5× SSC, 0.025 M sodium phosphate (pH 7.4), sonicated calf thymus DNA (50 mg/ml), 0.1% sodium dodecyl sulfate, and 50% (vol/vol) formamide. Blots were hybridized with cDNA probes in the same buffer mixture overnight at 42°C and washed at a final temperature of 60°C, in 0.1× SSC. Exposure was at −70°C on x-ray films (X-OMAT AR, Eastman Kodak, Rochester, N.Y.) with a single intensifying screen.

Anti-RyR Antibodies

A sequence-specific antibody (anti-RyR 8) was prepared against a synthetic peptide corresponding to a known ryanodine receptor amino acid sequence (20 amino acids, 4,681–4,700).23 C-LEFDGLYITEQPGID-DVDKGQ. A cysteine was added to the N-terminal end so that it could be specifically linked to keyhole limpet hemocyanin (Calbiochem, La Jolla, Calif.) via lysines using the bifunctional agent m-maleimidobenzoyl-N-hydroxysuccinimide ester. The antigen was injected into rabbits using the protocol of Gonatas et al.24 Affinity-purified antibody was prepared from the antiserum.25 The sequence was selected because of its similarity to the heart RyR (86% identity) and was found to react strongly with dog heart RyR by dot blot and Western blot analyses.

The specificity of the resulting anti-RyR 8 antibody was determined by immunoblots of purified skeletal RyR, skeletal terminal cisternae, and cultured bovine aortic endothelial cell membrane preparations. Membrane preparations were isolated as follows: tissues were pulverized using approximately 1 g/10 ml buffer of frozen
tissue sample with a mortar and pestle kept cold with dry ice and added to homogenization buffer (10 mM Tris-Cl, pH 8.0, 20 μg/ml leupeptin, 20 μg/ml aprotonin, 2 mM phenylmethylsulfonyl fluoride, and 3.6 mg/ml iodoacetamide) on ice. Tissue culture samples were simply scraped and added to homogenization buffer. Samples were added to ≈10 ml homogenization buffer and placed on ice for 8–10 minutes with frequent gentle mixing. Samples were homogenized in a Waring blender (pulsed for 30 seconds to 1 minute) at high speed. Ten microliters of 1 M NaCl/ml and 5 μl of 1 M MgCl2/ml were added, and the homogenates were mixed by inversion and left on ice. Homogenates were centrifuged at 1,000 rpm for 1–3 minutes in a low-speed centrifuge (Beckman Instruments, Inc., Fullerton, Calif.) to pellet debris. Homogenate (5 ml) was layered over a 10-ml cushion of 45% sucrose in 10 mM Tris-Cl, pH 8. Samples were centrifuged for 20 minutes at 6,500 rpm in an SW28 rotor. The supernatant was discarded, and the membrane was added to 10 mM Tris-Cl buffer and gently inverted to mix. The homogenate was pelleted by centrifugation at 10,000 rpm for 10 minutes in an SW28 rotor. The pellet was resuspended in 1 ml homogenization buffer and stored at −80°C. Terminal cisternae and RyR were purified as previously described.26,27 Protein samples were electrophoresed through 15% sodium dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose; immunoblots were performed with anti-RyR 8 antibody (1:5,000 dilution) as described previously.28 Antibody detection was with an Immun-Lite chemiluminescent assay kit (BioRad Laboratories, Richmond, Calif.) as per the manufacturer’s protocol.

A second antibody (anti-RyR 5) was prepared (as above) to a 22–amino acid synthetic peptide from the skeletal muscle ryanodine receptor (C-ENLRRSAGG-WGEAEGKEGTA), which corresponds to positions 1,333–1,353 of the published sequence.23 An N-terminal cysteine was added for linkage to keyhole limpet hemocyanin. This sequence was chosen because it shares only 18% sequence homology with the cardiac isoform of the ryanodine receptor protein.

Laser Scanning Confocal Microscopy

Confocal images were obtained with a Zeiss Axiowert 35-BioRad MRC-600 laser scanning confocal imaging system equipped with a krypton-argon laser. The laser was fitted with either a blue (excitation maximum, 488 nm) or a yellow (excitation maximum, 568 nm) filter block.

Electron Microscopy

Samples of aorta and cardiac ventricle were fixed in 2% glutaraldehyde and postfixed in osmium ferrocyanide to selectively stain the ER.29 These procedures were followed by plastic embedding in Spurr’s resin and sectioning. Micrographs were obtained on a Philips CM-12 microscope.

Results

Antibody Specificity

Western blots performed with anti-RyR 8 antibody on purified skeletal muscle RyR, skeletal muscle terminal cisternae, rabbit cardiac and skeletal muscle, and bovine aortic endothelial cell crude membrane preparations demonstrated immunodetection of a single high molecular weight species (molecular mass, ≈400,000,000) consistent with the RyR. Figure 1 is a Western blot of crude rabbit heart homogenate, bovine endothelial cell homogenate, purified rabbit cardiac RyR, and 125I-labeled myosin (as a molecular weight marker). One molecular weight species is demonstrated in the rabbit heart and endothelial cell homogenates, which correspond to the position of the purified cardiac RyR. No other bands were detected in the crude cardiac or endothelial cell homogenates using this antibody, indicating the specificity of the antibody for the cardiac (and skeletal muscle [not shown in Figure 1]) form of the RyR.

Western blots of antibody 5 (which was produced toward a skeletal muscle–specific epitope of the RyR) showed immunoreactivity to a single band on size-fractionated proteins from skeletal muscle, which is consistent with the RyR protein. There was no detectable cross hybridization to proteins from cardiac muscle (Y. Kijima, A. Timerman, and S. Fleischer, Vanderbilt University, unpublished observations).

Immunofluorescent Localization of Anti-RyR Antibody Binding Sites

The intracellular binding of anti-RyR antibody (anti-RyR 8) is seen in Figures 2 and 3. Laser-scanning confocal micrographs repeatedly demonstrated abundant TRITC fluorescence in both aortic and cardiac

FIGURE 1. Immunoblot demonstrating the specificity of the anti–ryanodine receptor antibody used for immunodetection of ryanodine receptors in endothelial cells. Lane 1, molecular weight marker (125I-labeled myosin); lane 2, purified cardiac ryanodine receptor (100 ng); lane 3, bovine aortic endothelial cell homogenate (50 μg); and lane 4, crude homogenate from rabbit heart (10 μg).
ventricular endocardial endothelium in each of the preparations examined (n=7). High magnification views revealed intense signals at one or both ends of the nucleus as well as brightly stained regions throughout the cells, illustrated in the through-focus series (Figure 2). A fluorescent signal was also detected in the perinuclear region when the focal plane passed through the center of the cell (Figure 2). Control preparations treated only with TRITC-conjugated donkey anti-rabbit F(ab')2 revealed no detectable staining at identical conditions of gain, black level, neutral density filter, confocal pinhole setting, and signal accumulation.

Subjacent vascular smooth muscle cells, displayed in Figure 3, demonstrate the difference in strength of fluorescent signal intensity when compared with the endothelium. Although not quantitative, these data argue for abundant antibody binding in endothelium, as compared with the signal in vascular smooth muscle or the myocardium under identical conditions of sample preparation and signal detection. The vascular endothelium of arterioles in the ventricular myocardium was also intensely fluorescent.

Confocal images of endothelium from ventricular endocardium and aorta showed only diffuse background staining with anti-RyR 5 compared with the more localized distribution of fluorescence observed with anti-RyR 8 labeling using identical conditions of sample preparation, immunolabeling, and signal detection. Anti-RyR 5 labeled guinea pig psoas muscle sections brightly.

**Rhodamine 123 Labeling of Endothelium**

Samples of fresh aortic and cardiac atrial tissue were stained with rhodamine 123 to demonstrate the pattern of fluorescence seen with selective mitochondrial labeling. The specificity of rhodamine 123 for mitochondria has previously been documented. Figure 4a demonstrates the pattern of fluorescence seen in atrial endocardial endothelium, and Figure 4b is representative...
FIGURE 4. Panel a: Photomicrograph of endocardial endothelium from guinea pig atrium labeled with rhodamine 123. The mitochondria fluoresce brightly with no significant staining of other intracellular structures. The pattern of intracellular labeling can be compared with images in Figures 2 and 3, where the tissue has been labeled with the anti–ryanodine receptor antibody and a fluorescent secondary antibody. Differences in the pattern and extent of fluorescent signal are apparent. Panel b: Confocal micrograph of unfixed aortic endothelium labeled with rhodamine 123. The internal elastic lamina exhibits bright autofluorescence at this excitation wavelength (488 nm). The mitochondria (arrow) are distributed around the nucleus of the endothelial cells as discrete, worm-like, brightly fluorescent objects. As in panel a, the perinuclear space, plasma membrane, and cytoplasm are not stained.

of that seen in aortic endothelium. Both images were collected at the same magnification as in Figure 2. The distribution and morphology of the fluorescent signal is quite different from that seen with anti-RyR labeling (Figure 2), because the latter presumably represents labeling of RyR peptides in the ER of the cell.

Electron Microscopy of Endothelium Treated With Osmium Ferrocyanide

Electron microscopy (Figures 5 and 6) revealed an extensive, darkly stained ER network in the guinea pig thoracic aortic endothelium. The ER is continuous with the nuclear envelope (see Figure 6) and is distributed throughout the cytoplasm, including regions at the ends of the nucleus. This network frequently approached the surface caveolae or the noncaveolar plasma membrane, suggestive of surface couplings at both the luminal and abluminal endothelial cell surfaces. The distribution of the ER is consistent with the confocal microscopic localization of anti-RyR antibody binding (Figures 2 and 3). Inspection of random multiple images showed that the fractional volume of the SR was smaller in the underlying vascular smooth muscle than in the aortic endothelium.

Northern Blot Analysis of Endothelial Cell RyR

Northern blot analyses performed on total RNA isolated from rabbit cardiac, bovine aortic endothelial cells, skeletal muscle, brain, and human umbilical vein...
endothelial cells (Figure 7) as well as smooth muscle (not shown) revealed a single, high molecular weight mRNA (=16 kb) present in the cardiac and smooth muscles, brain, and both types of endothelial cells. This 16-kb mRNA corresponds in size to the cardiac isoform of the RyR. This probe did not hybridize to the skeletal muscle isoform (lane 1, Figure 7).

**Discussion**

We have demonstrated prominent staining of vascular and endocardial endothelial cells by an antibody generated to a skeletal muscle epitope (C-LEFDGLY-ITEOPGDDDVKGQ), which crossreacts with the cardiac muscle RyR, and confirmed the expression of RyRs by Northern blot analysis of endothelial cell RNA. The light microscopic distribution of endothelial immunofluorescence was compatible with that of the ER, as shown by electron microscopy of conventionally fixed9 and osmium ferrocyanide-infiltrated (present study) endothelium. The staining of the perinuclear space by the anti-RyR antibody is also consistent with the continuity of this space with the ER. The distribution of reactive sites is unlikely to be mitochondrial, as demonstrated by the differences between the pattern of fluorescence seen in tissue specimens labeled with rhodamine 123 and anti-RyR 8. The fluorescent antibody was also excluded from the nuclear matrix, an otherwise common site of nonspecific antibody binding. These findings indicate that the endothelial ER, in addition to possessing a Ca$^{2+}$-ATPase30 that can accumulate divalent cations10 and release Ca$^{2+}$ through inositol 1,4,5-trisphosphate–gated channels,8,31 also contains a protein having homology to the RyR/Ca$^{2+}$-release channel of muscle. Unlike the skeletal RyR, which is localized to the triad regions of the sarcoplasmic reticulum and thought to be under voltage control of the transverse tubule dihydropyridine receptors, the RyRs of the endothelial cells are spread over the ER network. The RyR detected in endothelial cells displays immuno–cross-reactivity with

**FIGURE 6.** Electron micrograph of aortic endothelium at higher magnification. The specimen has been postfixed with osmium ferrocyanide to enhance staining of the endoplasmic reticulum. Arrows mark representative endoplasmic reticulum, and the double arrows indicate the continuity of the endoplasmic reticulum with the perinuclear envelope.

**FIGURE 7.** Northern blot analysis of ryanodine receptor. Lane 1, rabbit skeletal muscle (20 μg); lane 2, human umbilical vein endothelial cells (30 μg); lane 3, bovine aortic endothelial cells (30 μg); lane 4, rabbit brain (20 μg); and lane 5, rabbit heart (10 μg). The amount of total RNA loaded in each lane is indicated in parentheses. Exposure was at −70°C with one intensifying screen for 14 days. Low molecular weight bands corresponding to the 28S and 18S positions represent nonspecific binding of the cDNA probe, which is visualized after the prolonged exposure time required to identify the bands in the endothelial cell preparations. To confirm the amount of RNA loaded, this Northern blot was hybridized separately to a cDNA probe encoding glyceraldehyde phosphate dehydrogenase. The intensity of the glyceraldehyde phosphate dehydrogenase signal (a single band at ≈1.5 kb) was consistent with the relative amounts of total RNA loaded for each lane (not shown).
an antibody that detects both the skeletal and cardiac isoforms; however, only background fluorescence is demonstrated when the endothelial cells are labeled with an antibody directed toward a skeletal muscle–specific epitope. Northern blot analysis, with a cDNA probe that distinguishes between these two isoforms of the RyR, demonstrated that the endothelial cell form is more homologous to the cardiac isoform than the skeletal isoform. Ryanodine-binding proteins, some containing extensive functional homology to the muscle RyR, have already been identified in a variety of nonmuscle cells.32–34

The function and identity of the putative endothelial RyR/Ca2⁺ channel and its mechanism of activation remain to be established. The RyRs of skeletal,35 cardiac,36 and smooth37 muscle can be activated by Ca2⁺, and Ca2⁺-induced Ca2⁺ release is thought to be the mechanism of normal cardiac excitation–contraction coupling.38 Ca2⁺-induced Ca2⁺ release in muscle is facilitated by the action of caffeine on the RyR.39 However, the published effects of caffeine on endothelial [Ca2⁺]i are, at best, marginal: caffeine releases very little Ca2⁺ from bovine aortic endothelial cells in the absence of extracellular Ca2⁺.40 and induces Ca2⁺ spiking in only some cell preparations in cultured human umbilical venous endothelial cells.39 Therefore, it is possible that the RyR in endothelial cells is an isozyme of the RyR similar to that found in mink lung epithelial cells when stimulated with transforming growth factor-β.40 This isozyme does not respond to caffeine.40 It may also represent a receptor that is regulated, for example, by phosphorylation.41 The occasional responses of endothelial cells to caffeine (see above) could also reflect the additional expression, at very low abundance, of a second muscle-type RyR in endothelial cells.39,42,43 Could conceivably also involve RyRs. Such oscillations, although recycling of intracellular Ca2⁺ is involved, also require the presence of extracellular Ca2⁺.39,42 and, consequently, could be the result of, or at least be influenced by, Ca2⁺-induced Ca2⁺ release. However, this and the more important question of the identity of the physiological messenger,44 if other than Ca2⁺, that opens the RyR/Ca2⁺-release channels in nonmuscle cells such as the endothelium remain to be determined.

Acknowledgments

The authors wish to thank Drs. Scott Buck and Robert Boucek for carrying out the injection protocol, Dr. Anthony Timerman for purifying the anti-RyR 8, Dr. Thomas Michel for providing bovine aortic endothelial cells, Dr. Paul Tempst for providing synthetic ryanoide receptor peptides, and Anne-Marie Brillardes and Mary Alice Spina for expert technical assistance.

References

28. Marks AR, Moore DD, Backslof DJ, Garmisch B, Goodman HM: Conservation of the DNA binding domain and other properties

Lesch et al. Endothelial Ryanodine Receptors 487
35. Endo M: Calcium release from the sarcoplasmic reticulum. Physiol Rev 1977;57:71–108
36. Fabiato A: Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J Gen Physiol 1985;85:247–289
42. Laskey RE, Adams DJ, Cannell M, Van Breemen C: Calcium entry-dependent oscillations of cytoplasmic calcium concentration in cultured endothelial cell monolayers. Proc Natl Acad Sci USA 1992;89:1690–1694
Anti-ryanodine receptor antibody binding sites in vascular and endocardial endothelium.
R E Lesh, A R Marks, A V Somlyo, S Fleischer and A P Somlyo

Circ Res. 1993;72:481-488
doi: 10.1161/01.RES.72.2.481

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/72/2/481