Biochimica et Biophysica Acta 939 (1988) 587–594
Elsevier

BBA 73928

Ryanodine binding to sarcoplasmic reticulum membrane; comparison between cardiac and skeletal muscle

Marek Michalak [a], Phillippe Dupraz [a] and Varda Shoshan-Barmatz [b]

[a] *Department of Pediatrics, University of Alberta, Edmonton, Alberta (Canada) and* [b] *Department of Biology, Ben Gurion University of the Negev, Beer Sheva (Israel)*

(Received 2 June 1987)
(Revised manuscript received 9 November 1987)

Key words: Ryanodine binding; Sarcoplasmic reticulum; Calcium channel

[^{3}H]Ryanodine binding to skeletal muscle and cardiac sarcoplasmic reticulum (SR) vesicles was compared under experimental conditions known to inhibit or stimulate Ca^{2+} release. In the skeletal muscle SR, ryanodine binds to a single class of high-affinity sites (K_d of 11.3 nM). In cardiac SR vesicles, more than one class of binding sites is observed (K_d values of 3.6 and 28.1 nM). Ryanodine binding to skeletal muscle SR vesicles requires high concentrations of NaCl, whereas binding of the drug to cardiac SR is only slightly influenced by ionic strength. In the presence of 5′-adenylyl imidodiphosphate (p[NH]ppA), increased pH, and micromolar concentration of Ca^{2+} (which all induce Ca^{2+} release from SR) binding of ryanodine to SR is significantly increased in skeletal muscle, while being unchanged in cardiac muscle. Ryanodine binding to skeletal but not to cardiac muscle SR is inhibited in the presence of high Ca^{2+} or Mg^{2+} concentrations (all known to inhibit Ca^{2+} release from skeletal muscle SR). Ruthenium red or dicyclohexylcarbodiimide modification of cardiac and skeletal muscle SR inhibit Ca^{2+} release and ryanodine binding in both skeletal and cardiac membranes. These results indicate that significant differences exist in the properties of ryanodine binding to skeletal or cardiac muscle SR. Our data suggest that ryanodine binds preferably to site(s) which are accessible only when the Ca^{2+} release channel is in the open state.

Introduction

The sarcoplasmic reticulum (SR) is a highly specialized membrane system in muscle cell, which regulates myoplasmic Ca^{2+} levels, thereby controlling muscle contraction and relaxation [1]. Release of Ca^{2+} from the SR triggers contraction while active Ca^{2+} reuptake by the SR produces relaxation [2]. SR Ca^{2+} uptake, which is mediated by a membrane-bound Ca^{2+}-ATPase, has been extensively studied in both cardiac and skeletal muscle [1–5]. On the other hand, the mechanism by which Ca^{2+} is released from SR is not well understood [6,7]. A number of experimental models of Ca^{2+} release have been studied, using both skinned muscle fibers and isolated SR vesicles [6,7]. The available data suggest that Ca^{2+}-induced Ca^{2+} release or depolarization of the SR membrane may be the most likely candidates for the physiological mechanism by which Ca^{2+} is released from

Abbreviations: SR, sarcoplasmic reticulum; DCCD, dicyclohexylcarbodiimide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Pipes, 1,4-piperazinediethanesulphonic acid; Mes, 4-morpholineethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid; p[NH]ppA, 5′-adenylyl imidodiphosphate.

Correspondence: M. Michalak, Department of Pediatrics, University of Alberta, 6-106 Clinical Sciences Building, Edmonton, Alberta, Canada T6G 2G3.

SR membrane [6,7]. Ca^{2+} release from the SR membrane may also be induced by other means, such as pH changes [8], sulfhydryl group reagents [9], heavy-metal ions [9,10], and adenine nucleotides [12,13]. Ca^{2+} release from the SR membrane can be effectively inhibited by Mg^{2+} and micromolar concentrations of ruthenium red [6,7].

Recent studies suggest that the cardiac and skeletal muscle SR has a ligand-gated Ca^{2+} release channel, which is influenced by ryanodine [14–16]. Ryanodine is a neutral plant alkaloid with profound effects on muscle [17]. It is a potent inhibitor of contractile tension development in cardiac muscle, whereas in vertebrate skeletal muscle, the drug produces irreversible contracture [17]. It has been suggested that ryanodine may interact with the Ca^{2+} release channel of the SR [16,18–23]. Purification of the ryanodine receptor from skeletal SR vesicles has been recently reported [21–23]. Solubilization of the ryanodine receptor from cardiac SR vesicles has also been reported [24].

Recent studies using radiolabelled ryanodine demonstrate that the drug binds to the SR isolated form skeletal or cardiac muscle [18–20]. Binding is Ca^{2+}-dependent, and is inhibited by ruthenium red or dantrolene. Preliminary comparison between ryanodine binding to cardiac and skeletal muscle SR have already been reported [19]. In this report we extend these observations by comparing ryanodine binding to cardiac and skeletal muscle SR vesicles under conditions known to influence Ca^{2+} release activity from SR membranes.

Experimental procedures

Materials. [^{3}H]Ryanodine was obtained from New England Nuclear, unlabelled ryanodine was a generous gift from Dr. K.P. Campbell of the University of Iowa, Iowa. Ruthenium red was from Fluke AG, Buchs, Switzerland. Dicyclohexylcarbodiimide (DCCD), Hepes, Pipes, Mes and p[NH]ppA were obtained from Sigma.

Isolation of SR membranes. Rabbit skeletal muscle SR was isolated and fractionated into heavy and light SR vesicles according to the method of Meissner [25]. Cardiac SR was isolated from sheep heart by the procedure of Chamberlain et al. [26], and subsequently fractionated on discontinuous sucrose gradient. Fraction banding at the 25%/28% and the 28%/36% sucrose interphases were collected as light and heavy cardiac SR vesicles, respectively. Protein concentration was determined according to Lowry et al. [27].

[^{3}H]Ryanodine binding assay. The heavy fraction of cardiac or skeletal muscle SR membranes (at a protein concentration of 2 mg/ml) was incubated with 20 nM [^{3}H]ryanodine (spec. act. = 55.3 Ci/mmol), unless otherwise specified, in a standard binding solution containing 0.5 M NaCl, 10 mM Pipes (pH 7.1) and 50 μM $CaCl_2$, for 2 h at 37°C. As indicated in some experiments, NaCl was substituted for 0.2 M sucrose. The unbound ryanodine was separated from the protein-bound ryanodine by filtration of protein aliquots (20–40 μg) through PHWP 0.3 μm Millipore filters, followed by washing three times with 5 ml of unlabelled buffer containing 0.2 M NaCl, 10 mM Pipes (pH 7.1) and 50 μM $CaCl_2$. The counts retained on the filters were determined using standard liquid scintillation counting techniques. Ca^{2+} dependence of ryanodine binding to SR vesicles was measured at different free Ca^{2+} concentrations using an Ca^{2+}-EGTA buffer. The free Ca^{2+} concentration was based on the EGTA association constant reported by Fabiato and Fabiato [28], and the calculation of free Ca^{2+} was accomplished by the use of a computer program. Specific binding of ryanodine is defined as the difference between total binding (with [^{3}H]-ryanodine alone) and nonspecific binding (with [^{3}H]ryanodine and 10 μM unlabelled ryanodine). Nonspecific binding was approx. 8–10% and 10–15% of a total binding in skeletal muscle and cardiac SR vesicles, respectively. Results are the average of duplicate samples which differed by 5–8%.

Results and Discussion

Ryanodine binding

Ryanodine binding to the heavy fractions of cardiac and skeletal muscle SR membrane is shown in Fig. 1. Scatchard analysis of specific ryanodine binding to skeletal muscle SR reveals a single class of high-affinity binding sites with a K_d of 11.3 nM (Fig. 1B). Cardiac SR, however, yields nonlinear Scatchard plots, indicating the presence of

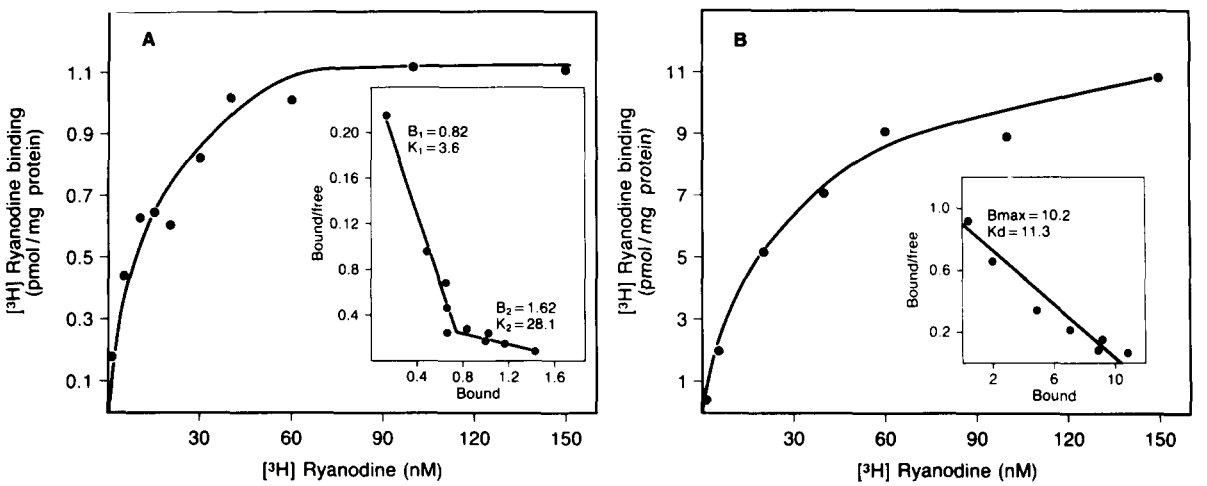

Fig. 1. Specific binding of [^{3}H]ryanodine to cardiac (A) and skeletal muscle (B) SR vesicles. Ryanodine binding was carried out as described under Experimental Procedures. For Scatchard insets, B_{max} and bound ligand are given as pmol/mg protein, and K_d as nM.

multiple binding sites having greatly different affinities for ryanodine (K_d values of 3.6 and 28.1 nM) (Fig. 1A). The B_{max} for skeletal muscle SR is 10-times that of cardiac SR. Under optimal Ca^{2+} concentration, binding equilibrium is reached within 2 h at 37°C (data not shown). The affinity of the receptor for ryanodine reported here, for both skeletal and cardiac SR, is much higher than that described previously [18,19]. This may result from differences in isolation of SR vesicles used by us and others [18,19]. Alternatively, it may also reflect differences in the conditions used for the ryanodine binding.

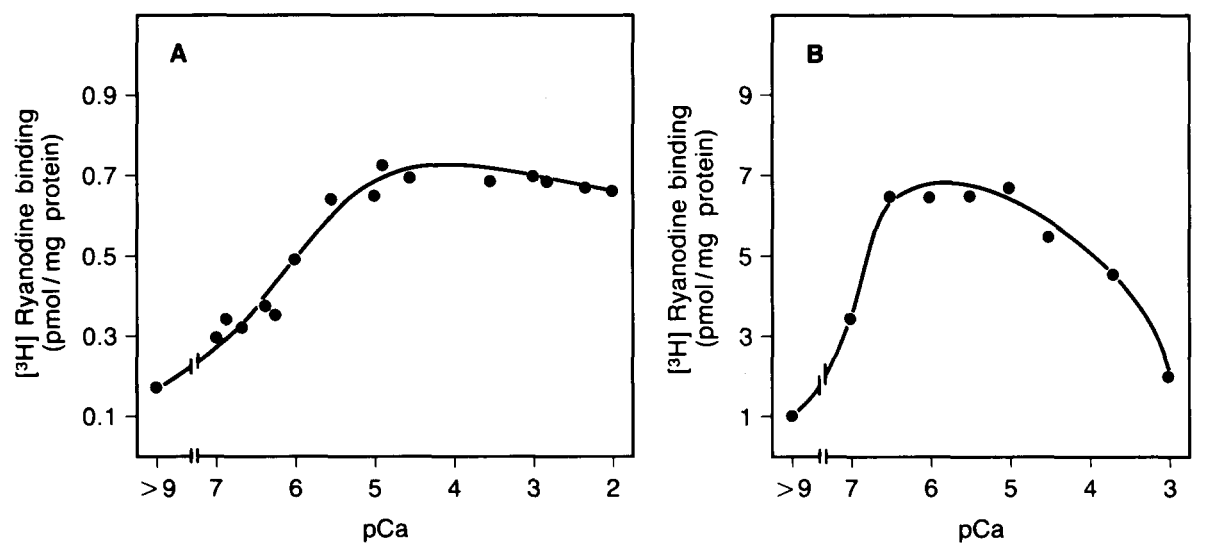

Fig. 2. Ca^{2+} dependence of ryanodine binding to cardiac (A) and skeletal muscle (B) SR vesicles. Specific binding of ryanodine to SR vesicles and determination of free Ca^{2+} concentration was as described under Experimental Procedures.

Effects of Ca^{2+} and Mg^{2+} on ryanodine binding and Ca^{2+} release

Binding of ryanodine to the SR membranes requires the presence of Ca^{2+}. Fig. 2 shows the Ca^{2+} dependence of ryanodine binding to both cardiac and skeletal muscle SR vesicles. The optimal concentrations of Ca^{2+} for ryanodine binding to skeletal muscle SR are in the range of 1–50 μM (Fig. 2B). Ryanodine binding to cardiac SR was maximal at 5 μM [Ca^{2+}], and in contrast to skeletal SR it did not decline at high Ca^{2+} concentrations (up to 5 mM). The dependence of ryanodine binding on Ca^{2+} concentration is consistent with the observations of Pessah et al. [19]. We, as well as Alderson and Feher [29], however, did not observe a sharp decline in ryanodine binding to cardiac SR vesicles at high Ca^{2+} concentrations, as reported by Pessah et al. [19].

Mg^{2+} at millimolar concentrations inhibits Ca^{2+} release from skeletal muscle SR and to the lesser extent from cardiac SR vesicles (Table I and Refs. 6, 7, 30 and 31). We, therefore, tested the effect of Mg^{2+} on ryanodine binding to cardiac and skeletal muscle SR vesicles. Ryanodine binding to skeletal muscle SR was sharply inhibited at millimolar concentrations of Mg^{2+}, while binding to cardiac SR was only slightly inhibited by Mg^{2+} concentrations up to 50 mM (Fig. 3). Inhibitory effects of Ca^{2+} and Mg^{2+} on ryanodine binding to skeletal muscle SR presented in Figs. 2 and 3 were not due to the change in the K_d of the binding but to the B_{max} only (data not shown). The results presented in Table I and Fig. 3 indicate a significant difference in the mechanism by which Mg^{2+} affects the ryanodine binding and Ca^{2+} release processes in these two muscle types. Combined with the observed effects of Mg^{2+} on Ca^{2+} release, they suggest that Mg^{2+} inhibition of ryanodine binding correlates with its inhibitory effects on Ca^{2+} release activity. The effect of Mg^{2+} on Ca^{2+} release from skeletal muscle SR has been extensively studied [6,7]. It is suggested that Mg^{2+} may compete with Ca^{2+} for a binding to the Ca^{2+} channel, thereby increasing the concentration of Ca^{2+} required to trigger Ca^{2+} release. Mg^{2+} may also block the Ca^{2+} channel, as shown by a Mg^{2+}-induced decrease in the maximal rate of Ca^{2+} release at optimum Ca^{2+} concentration.

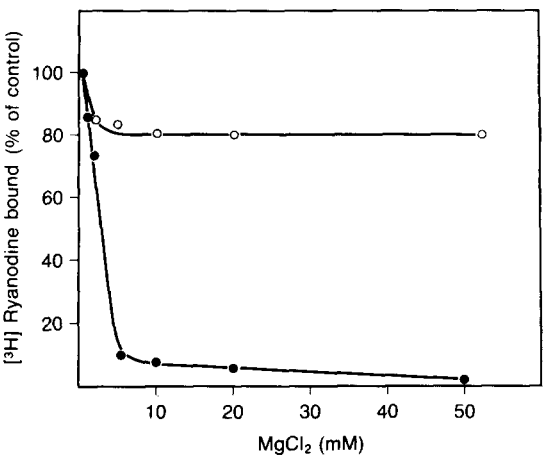

Fig. 3. Ryanodine binding to cardiac (○) and skeletal muscle (●) SR vesicles as a function of different Mg^{2+} concentrations. Ryanodine binding to SR vesicles was carried out as described under Experimental Procedures at different Mg^{2+} concentrations. 100% corresponds to binding in the absence of Mg^{2+}.

TABLE I

EFFECT OF Mg^{2+} AND RUTHENIUM RED ON ALKALINE pH-INDUCED Ca^{2+} RELEASE FROM CARDIAC OR SKELETAL SR VESICLES

SR membranes (5 mg/ml) were passively loaded with $^{45}CaCl_2$ in a solution containing 20 mM Mops (pH 6.8), 100 mM KCl and 5 mM $^{45}CaCl_2$, ($5 \cdot 10^7$ cpm/ml) for 2 h at 22°C. Ca^{2+} efflux was assayed by a 50-fold dilution of the loaded vesicles into a solution containing 100 mM KCl, 1 mM EGTA and 20 mM Mops (pH 6.8) or 20 mM Tricine (pH 8.0) and the compounds indicated in the table. Ca^{2+} content was determined 20 s after dilution by filtering 200 μl samples through 0.3 μm nitrocellulose filters, and washing with 3 ml of a solution identical to the one used for dilution of the vesicles. Radioactivity on the filters was measured in a liquid scintillation counter. Ca^{2+} content of the vesicles was 25.5 and 40 nmol/mg protein for cardiac and skeletal membranes, respectively.

Condition	Ca^{2+} release (nmol/mg protein)	
	cardiac SR vesicles	skeletal SR vesicles
pH 6.8	4.0	4.0
pH 6.8 + Mg^{2+} (5 mM)	4.0	4.0
pH 6.8 + ruthenium red (20 μM)	3.5	3.0
pH 8.0	15.5	27.0
pH 8.0 + Mg^{2+} (5 mM)	13.8	4.0
pH 8.0 + ruthenium red (20 μM)	1.4	4.0

DCCD inhibition of Ca^{2+} release and ryanodine binding

DCCD is a potent inhibitor of Ca^{2+} release from SR vesicles [8,33,34]. Table II shows that DCCD modification of cardiac and skeletal SR vesicles inhibits both Ca^{2+} release and [^{3}H]ryanodine binding in both membranes. Ryanodine binding was inhibited to a greater degree than Ca^{2+} release activity (Table II). This may be due to the residual low concentrations of DCCD still present during the ryanodine-binding assay. DCCD inhibition of Ca^{2+} release induced by either alkaline pH or by external Ca^{2+} was demonstrated previously for skeletal [8,32] and cardiac [34] SR vesicles. The inhibitory effect of DCCD on ryanodine binding may be due to its blockage of Ca^{2+} release channel [8,33,34]. This would support our concept that ryanodine binding requires an open channel state. However, it is also possible that DCCD inhibition of ryanodine binding may be a result of its direct effect on the ryanodine-binding site.

TABLE II

EFFECT OF DCCD – MODIFICATION OF SR MEMBRANES ON Ca^{2+} RELEASE AND [^{3}H]RYANODINE-BINDING ACTIVITIES

Cardiac or skeletal SR membranes (1 mg/ml) were incubated without (control) or with DCCD at 22°C in a solution containing 20 mM Mes (pH 6.1), 100 mM KCl and 0.5 mM EGTA. After 30 min, the membranes were collected by centrifugation at 100000 × *g* for 20 min and the pellets were resuspended in a solution containing 20 mM Mops (pH 6.8) and 100 mM KCl at a final concentration of 10 mg protein/ml. Ca^{2+} release activity was measured as described in Table I. Ca^{2+} content of the passively loaded vesicles (100%) was 66 and 78 nmol/mg protein for cardiac and skeletal SR vesicles, respectively. [^{3}H]Ryanodine binding was assayed as described under Experimental Procedures. Ryanodine binding (100%) was 4.4 and 2.9 pmol/mg protein for cardiac and skeletal membranes, respectively.

Membrane	Ca^{2+} released per 30 s (% of control)	[^{3}H]Ryanodine binding (% of control)
Cardiac SR		
control	85	100
treated with DCCD (25 μM)	64	43
treated with DCCD (50 μM)	44	22
Skeletal SR		
control	75	100
treated with DCCD (25 μM)	50	37
treated with DCCD (50 μM)	31	20

Effects of NaCl and pH

The effect of different NaCl concentration on ryanodine binding to skeletal and cardiac muscle SR is shown in Fig. 4. Almost no binding of ryanodine to skeletal SR was obtained in the absence of NaCl. Under the same conditions, ryanodine binding to cardiac SR was about 80% of that observed in the presence of NaCl, indicating that NaCl had little effect on ryanodine binding to cardiac SR. The effects of monovalent cation on ryanodine binding are not clear. It may stimulate the binding either indirectly, by screening fixed negative charges present in SR membranes [34] thereby affecting the ryanodine receptor, or directly by interacting with the receptor and stabilizing the ryanodine-receptor complex.

An increase of the pH of the external medium stimulates Ca^{2+} release from skeletal [8] and cardiac SR vesicles (Tables I and II). The effects of increasing pH on ryanodine binding to SR vesicles is shown in Table III. Ryanodine binding to both skeletal muscle and cardiac SR was increased as pH of the binding medium increased.

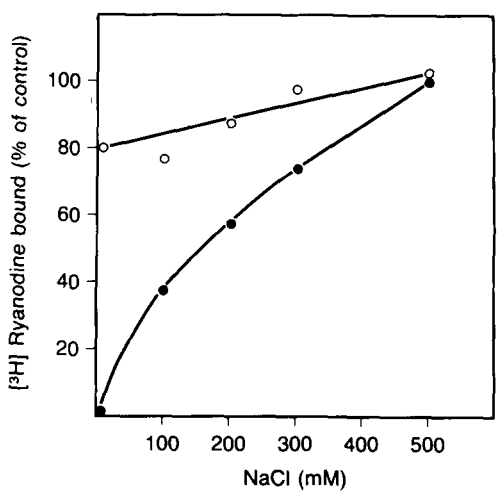

Fig. 4. NaCl concentration dependence of ryanodine binding to cardiac (○) and skeletal muscle (●) SR vesicles. Ryanodine binding to SR vesicles was carried out as described under Experimental Procedures at different NaCl concentrations. At low NaCl concentrations (0–100 mM), 0.2 M sucrose was included in the binding medium. 100% corresponds to binding at 500 mM NaCl.

Effects of p[NH]ppA

Adenine nucleotides profoundly affect Ca^{2+} release from SR membranes by increasing the rate of Ca^{2+}-induced Ca^{2+} release [6,7]. We, therefore, tested the effects of p[NH]ppA on ryanodine binding to SR vesicles. Increasing the concentration of p[NH]ppA resulted in a significant increase of ryanodine binding to skeletal muscle SR, but had no effect on ryanodine binding to cardiac SR (Fig. 5). Recently, Campbell et al. [22] reported increased ryanodine binding in the presence of ATP to the immuno-precipitated ryanodine-binding protein from skeletal muscle SR. This effect of ATP cannot be due to ATP hydrolysis and/or protein phosphorylation, since the stimulation of ryanodine binding is observed in the presence of nonhydrolyzable analog of ATP (p[NH]ppA) (Fig. 5). The involvement of adenine nucleotides in Ca^{2+} release has been suggested for both skeletal and cardiac SR [5,6,12,13,30]. Adenine nucleotides, in millimolar concentrations, enhance the rate of Ca^{2+} release from cardiac and skeletal muscle SR vesicles being more effective in stimulating Ca^{2+} release from skeletal than cardiac SR vesicles [30,31].

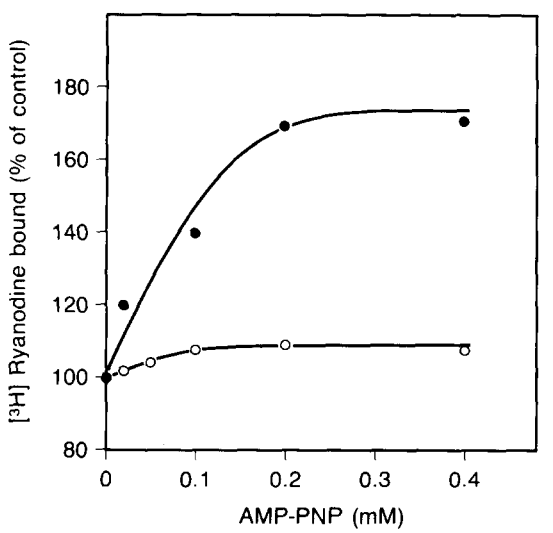

Fig. 5. Ryanodine binding to cardiac (○) and skeletal muscle (●) SR vesicles in the presence of varying concentration of p[NH]ppA (AMP-PNP). Ryanodine binding to SR vesicles was carried out as described under Experimental Procedures. Free Ca^{2+} concentration was kept at 50 μM, taking into account Ca^{2+} bound to p[NH]ppA. 100% corresponds to binding in the absence of p[NH]ppA.

TABLE III

EFFECT OF INCUBATION pH ON RYANODINE BINDING

SR membranes (2 mg/ml) were incubated with 20 nM [^{3}H]ryanodine for 2 h at 37 °C. Bound ryanodine was determined by Millipore filtration as described under Experimental Procedures. At pH values from 6.1 to 7.5 and 8.0 to 8.6, 20 mM Pipes or 20 mM Tris were used, respectively. The binding at pH 7.1 was taken as 100%.

pH	[^{3}H]Ryanodine binding (pmol/mg protein)	
	cardiac SR	skeletal muscle SR
6.1	0.61 (33%)	0.33 (12%)
6.5	1.50 (80%)	1.85 (68%)
7.1	1.87 (100%)	2.70 (100%)
7.5	1.64 (88%)	2.76 (102%)
8.0	1.78 (98%)	3.09 (114%)
8.6	2.04 (109%)	3.05 (113%)

In this report, ryanodine binding to cardiac and skeletal muscle SR vesicles was compared under different experimental conditions. The ryanodine receptor is believed to be related to the SR Ca^{2+} release channel [14–23]. We therefore, investigated ryanodine binding in the presence of Mg^{2+}, Ca^{2+}, p[NH]ppA, different pH values, and in DCCD-modified membranes, all of which are known to affect Ca^{2+} release from the SR membrane [6–8,32,33]. The binding of ryanodine to SR membranes appears to be dependent on whether the Ca^{2+} release channel is present in an open or closed configuration. It is demonstrated in this study, that conditions which induce opening of the SR Ca^{2+} channel, such as the presence of adenine nucleotides, elevated pH (> 6.8), and micromolar Ca^{2+} concentrations, lead to an increased ryanodine binding to cardiac and skeletal muscle SR. On the other hand, ryanodine binding to the SR vesicles is strongly inhibited by conditions that inhibit Ca^{2+} release, such as high Ca^{2+} concentrations, millimolar Mg^{2+} concentrations, low pH (less than 6.8), micromolar concentrations of ruthenium red and DCCD modification of the membranes. These results indicate that ryanodine may be binding to site(s) which are accessible only in the open state of the Ca^{2+} release channel.

The differences in the class of binding sites,

and inhibitory effects of Mg^{2+} and Ca^{2+} on ryanodine binding, between cardiac and skeletal muscle SR vesicles reproted in this work indicate that not only the ryanodine receptor-Ca^{2+} channel system(s), but also the mechanism for the release of Ca^{2+} from cardiac and skeletal muscle SR may be different. This correlates well with several important differences observed in the extent to which the cardiac and skeletal muscle Ca^{2+} channels are activated by Ca^{2+} and adenine nucleotide and inhibited by Mg^{2+} [30,31]. As reported recently by Meissner [30,31], micromolar concentrations of Ca^{2+} stimulate Ca^{2+} release in the absence of Mg^{2+} and nucleotides to as much as 50% of the optimal rate, whereas only 1–3% of the optimal rate could be achieved in skeletal vesicles in the absence of nucleotide and Mg^{2+} [31]. In contrast, adenine nucleotides are more effective in stimulating Ca^{2+} release from skeletal SR, as opposed to cardiac SR vesicles. This difference is particularly apparent at low Ca^{2+} concentrations. Ca^{2+} efflux from cardiac vesicles is also less sensitive to Mg^{2+} inhibition than from skeletal vesicles (Table I and Ref. 30).

In cardiac muscle, earlier ultrastructural studies [35] indicate a correlation between the sensitivity of the muscle to ryanodine and the presence of a transverse tubular system. Moreover, it has been suggested [35] that ryanodine inhibits contraction in heart muscle by dissociating the T-tubules from the SR and thus, uncoupling excitation from contraction. This is in agreement with the recent observation that ryanodine may be binding to the 'feet' structures of skeletal SR membrane [21,22]. The relationship between the 'feet' structure, ryanodine receptor and Ca^{2+} release channel in SR membrane has not been clearly determined. Different properties of the ryanodine-binding site(s) in heavy SR isolated from the two types of muscle reported here, as well as its different effects on skeletal and cardiac muscle [17], may be related to the ultrastructural differences between these two types of muscle [36].

In conclusion, our data indicates that there are significant differences in ryanodine binding to cardiac and skeletal muscle SR vesicles. In addition, not only the ryanodine receptor and/or Ca^{2+} channel, but also Ca^{2+} release mechanisms may be significantly different in these two muscle types. Clarification of these points will require further studies on the Ca^{2+} release mechanism in cardiac SR membrane, as well as isolation and characterization of the functional ryanodine receptor/Ca^{2+} release channel from both skeletal and cardiac SR membrane.

Acknowledgments

We thank Dr. D.H. MacLennan for providing space in his laboratory to carry out this work. We thank Dr. K.P. Campbell of the University of Iowa for providing ryanodine and Dr. G.D. Lopaschuk for help during the preparation of this manuscript. This work was supported by the Ontario Heart and Stroke Foundation and the Alberta Heart and Stroke Foundation. M.M. is a scholar of the Medical Research Council and the Alberta Heritage Foundation for Medical Research.

References

1 Michalak, M. (1985) in The Enzymes of Biological Membranes (Martonosi, A.N., ed.), Vol. 3, pp. 115–155, Plenum Press, New York.
2 Tada, M., Yamamoto, T. and Tonomura, Y. (1978) Physiol. Rev. 58, 1–79.
3 Moller, J.Y., Andersen, J.P. and LeMaire, M. (1982) Mol. Cell. Biochem. 42, 83–107.
4 Tada, M., Yamada, M., Kadoma, M., Inui, M. and Ohmori, F. (1982) Mol. Cell. Biochem. 46, 83–107.
5 Ikemoto, N. (1982) Annu. Rev. Physiol. 44, 297–317.
6 Martonosi, A.N. (1984) Physiol. Rev. 64, 1240–1320.
7 Endo, M. (1985) Curr. Top. Membr. Transp. 25, 181–230.
8 Shoshan, V., MacLennan, D.H. and Wood, D.S. (1981) Proc. Natl. Acad. Sci. USA 78, 4828–4832.
9 Bindoli, A. and Fleischer, S. (1983) Arch. Biochem. Biophys. 221, 458–466.
10 Trimm, J.L., Salama, G. and Abramson, J.J. (1986) J. Biol. Chem. 261, 16092–16098.
11 Abramson, J.J., Trimm, J.L., Weden, L. and Salama, G. (1983) Proc. Natl. Acad. Sci. USA 80, 1526–1530.
12 Kirino, Y., Osakabe, M. and Shimizu, H. (1983) J. Biochem. 94, 1111–1118.
13 Ohnishi, S.T. (1979) J. Biochem. 86, 1147–1150.
14 Meissner, G. (1986) J. Biol. Chem. 261, 6300–6306.
15 Feher, J.J. and Lipford, G.B. (1985) Biochim. Biophys. Acta 813, 77–86.
16 Lattanzio, F.A., Jr., Schlatterer, R.G., Nicar, M., Campbell, K.P. and Sutko, J.L. (1987) J. Biol. Chem. 262, 2711–2718.
17 Henden, D.J. and Fairhurst, A.S. (1969) Pharmacol. Rev. 21, 1–25.

18 Fleischer, S., Ogunbummi, E.M., Dixon, M.C. and Fleer, E.A.M. (1985) Proc. Natl. Acad. Sci. USA 82, 7256–7259.
19 Pessah, J.N., Waterhouse, A.L. and Casida, J.E. (1985) Biochem. Biophys. Res. Commun. 128, 449–456.
20 Pessah, J.N., Francini, A.O., Scales, D.J., Waterhouse, A.L. and Casida, J.E. (1986) J. Biol. Chem. 261, 8643–8648.
21 Inui, M., Saito, A. and Fleischer, S. (1987) J. Biol. Chem. 262, 1740–1747.
22 Campbell, K.P., Knudson, C.M., Imagawa, T., Leung, A.T., Sutko, J.L., Kahl, S.D., Raab, C.R. and Madson, L. (1987) J. Biol. Chem. 262, 6460–6463.
23 Lai, F.A., Erickson, H., Block, B.A. and Meissner, G. (1987) Biochem. Biophys. Res. Commun. 143, 704–709.
24 Seifert, J. and Casida, J.E. (1986) Biochim. Biophys. Acta 861, 399–405.
25 Meissner, G. (1984) J. Biol. Chem. 259, 2365–2374.
26 Chamberlain, B.K., Levitsky, D.D. and Fleischer, S. (1983) J. Biol. Chem. 258, 6602–6609.
27 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
28 Fabiato, A. and Fabiato, F. (1979) J. Physiol. Lond. 45, 463–505.
29 Alderson, B.H. and Feher, J.J. (1987) Biochim. Biophys. Acta 900, 221–229.
30 Meissner, G. and Henderson, J.S. (1987) J. Biol. Chem. 262, 3065–3073.
31 Meissner, G., Darling, E. and Eveleth, J. (1986) Biochemistry 25, 236–244.
32 Yamamoto, N. and Kasai, M. (1982) J. Biochem. 92, 485–496.
33 Chamberlain, B.K., Volpe, P. and Fleischer, S. (1984) J. Biol. Chem. 259, 7547–7553.
34 Arrio, B., Johannin, G., Carrette, A., Chevallier, J. and Brethes, D. (1984) Arch. Biochem. Biophys. 228, 220–229.
35 Penefsky, Z.J. (1974) Pflugers Arch. 347, 185–198.
36 Walker, S.M., Schrodt, G.R. and Edge, M.B. (1971) J. Anat. (Lond.) 108, 217–230.