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How Many Cysteine Residues Regulate Ryanodine Receptor Channel Activity?

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ABSTRACT

RyRs contain 80–100 cysteine residues per subunit, of which ~25% are free for covalent modification, while the remainder are either modified or form intraprotein disulfides. Oxidizing and nitrosylating reagents have several effects on single RyR channel activity, which depend on the type of modifying reagent, the isoform of the RyR, and ligands bound to the channel. We present evidence here for four major classes of functional cysteine residues associated with RyR channels, *i.e.*, two classes with free -SH groups that either activate or inhibit channels when covalently modified and two classes, with endogenous modification, that either inhibit or activate. Single-channel characteristics provide evidence for four discrete responses within the first activating class, two responses within the second inhibiting class and two types of response within the third endogenously modified class. All but one of these changes in channel properties depend on residues located on the cytoplasmic or membrane-associated domains of the RyR; the remaining response is confined to the luminal domain. If it is assumed that each type of response depends on a separate subclass of cysteine residue and that each subclass contains a minimum of one cysteine per subunit, our results suggest that there are at least nine cysteine residues per subunit with functional connections to the gating mechanism of RyR channels. These cysteine residues may be selectively modified under physiological and pathological conditions to regulate Ca^{2+} release from the sarcoplasmic reticulum and contraction. Antiox. Redox Signal. 2, 27–34.

INTRODUCTION

RYANODINE RECEPTORS (RyRs) are essential ion channels that release Ca^{2+} from intracellular stores during contraction in striated muscle and Ca^{2+} signaling in cells like neurons and lymphocytes (Dulhunty *et al.*, 1996; Guse *et al.*, 1999). RyRs are regulated by many ligands and contain ~80–100 cysteine residues per subunit, of which ~20 are free for modification by oxidation, nitrosylation, or alkylation (Xu *et al.*, 1998), and can activate or inhibit RyR channels, Ca^{2+} release from sarcoplasmic reticulum (SR) and contraction (Abramson and Salama, 1989; Oba *et al.*, 1996a; Eager *et al.*, 1997; Reid, 1998) and are likely to modulate Ca^{2+} regulation *in vivo*, under physiological and pathological conditions.

Because covalent modification of cysteines can have different actions on RyR activity, depending on the nature and concentration of reagents and background channel activity, different classes of functional cysteines are thought to exist (Aghdasi *et al.*, 1997a,b; Eager and Dulhunty, 1998, 1999; Xu *et al.*, 1998). Reagents discussed here are (i) common reactive disulfides, -dithiodipyridines (4,4'-DTDP) or 5,5'-dithio-*bis*(2-nitrobenzoic acid) (DTNB),

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(ii) a novel reactive disulfide, gliotoxin (GTX), (iii) an organomercurial, thimerosal, and (iv) *S*-nitroso-*N*-acetyl-penicillamine (SNAP), a donor of nitric oxide (NO). Reactive disulfides are thiol-specific, whereas thimerosal and NO act preferentially on thiols (Sayers *et al.*, 1993; Simon *et al.*, 1996; Xu *et al.*, 1998). The effects of all reagents are prevented or reversed by dithiothreitol (DTT), confirming thiol involvement.

Usually, it is not clear whether oxidizing/nitrosylating reagents form stable mixed disulfides (or nitrosothiols) with cysteines, or whether further oxidation forms intraprotein disulfides. For example, it was thought that nitrosothiols were unstable and *S*-nitrosylation was followed by oxidation to disulfides. This is now known to be wrong, because stable nitrosothiols exist in several proteins including RyRs (Simon *et al.*, 1996; Xu *et al.*, 1998). Some reagents can at the same time *S*-nitrosylate and oxidize different thiols (Xu *et al.*, 1998), and oxidation and *S*-nitrosylation can occur without altering RyR activity—*i.e.*, not all free –SH groups are linked to the channel's "gating" mechanisms (Aghdasi *et al.*, 1997a,b; Xu *et al.*, 1998).

FUNCTIONALLY ACTIVE CYSTEINE RESIDUES

Because oxidizing (nitrosylating and alkylating) or reducing reagents can modify RyR activity (Aghdasi *et al.*, 1997a,b; Xu *et al.*, 1998; Tables 1 and 2), functionally active cysteines exist with both free –SH and modified (–S-R–) groups (Fig. 1).

RyRs respond to oxidizing and nitrosylating agents with increased activity, which can be followed by inhibition with higher concentrations of reagents or prolonged exposures (Eager *et al.*, 1996; Eager and Dulhunty, 1998; Table 1). This suggests two classes of free –SH groups that, when oxidized, either activate or inhibit RyRs (Fig. 1). It is unlikely that modification of one class is followed by sequential changes in activity, because (i) activation occurs without inhibition with <1 m*M* 4,4′-DTDP, thimerosal, or GTX (Eager *et al.*, 1997; Marengo *et al.*, 1998; Table 1), (ii) activation is followed by inhibition with 1 m*M* 4,4′-DTDP or thimerosal, but not with 1 m*M* DTNB or SNAP (Table 1), and (iii) inhibition can occur without initial activation (Eager and Dulhunty, 1998; Haarmann *et al.*, 1999; Hart and Dulhunty, 1999).

TABLE 1. ACTIVATION AND INHIBITION BY OXIDIZING AND NITROSYLATING REAGENTS

		Activation					*Inhibition*		
							Reversibility		
Reagent		P_o	T_o *long*	T_o *v.long*	F_o	*Reversal with DTT*	ΔV	*DTT*	*Prevention by DTT*
Cardiac RyRs[a]									
1 m*M* 4,4′-DTDP	*cis*	↑ ↑	↑ ↑	↑ ↑	↑	Yes	No	No	Yes
	trans	↑ ↑	↑ ↑	—	↑	Yes	No	No	Yes
1 m*M* thimerosal	*cis*	↑ ↑	↑ ↑	—	↑	Yes	No	No	Yes
	trans	↑ ↑	↑ ↑	—	↑	Yes	No	No	Yes
Skeletal RyRs									
1 m*M* 4,4′-DTDP	*cis*[b]	↑ ↑	↑ ↑	—	—	Yes	No	Yes	nt
1 m*M* DTNB	*cis*[b]	↑ ↑	↑ ↑	—	—	Yes	ni —		—
	trans	↑ ↑	↑ ↑	—	—	Yes	ni —	—	—
200 μ*M* GTX	*cis*[c]	↑ ↑	—	—	↑ ↑	Yes	ni —	—	—
	trans	↑ ↑	—	—	↑ ↑	Yes	ni —	—	—
10 μM SNAP	*cis*[d]	↑ ↑	—	—	↑ ↑	Yes	ni —	—	—
1 m*M* SNAP	*cis*[d]	na	↑ ↑	—	↓ ↓	nt	ni —	—	—
1 m*M* SNAP (to ATP-activated channels	*cis*[d]	na	—	—	—	—	Yes	Yes	nt

na, no activation; ni, no inhibition; nt, not tested.
[a]Eager *et al.* (1998, 1999).
[b]Haarmann *et al.* (1999).
[c]Green, Pace, Hurne, Waring, Hart, and Dulhunty, unpublished.
[d]Hart and Dulhunty (1999).

TABLE 2. EFFECTS OF REDUCING REAGENTS ON RyR CHANNEL ACTIVITY

Reagent	*Activation* P_o	*Inhibition* P_o	*Reversibility with DTNB*
Cardiac RyRs			
cis GSH (5 m*M*)[a]	na	↓ ↓	nt
cis DTT (10 m*M*)[a]	na	↓ ↓	nt
Skeletal RyRs[a]			
cis DTT (2 m*M*)[b,c]	na	ni	
cis DTT (10 m*M*)[b]	↑ ↑	ni	Yes
trans DTT (2 m*M*)[c]	↑ ↑	ni	nt
trans DTT (10 m*M*)[b]	↑ ↑	ni	Yes

na, no activation; ni, no inhibition; nt, not tested.
[a]Eager *et al.* (1997).
[b]Haarmann *et al.* (1999).
[c]Green, Pace, Hurne, Waring, Hart, and Dulhunty, unpublished.

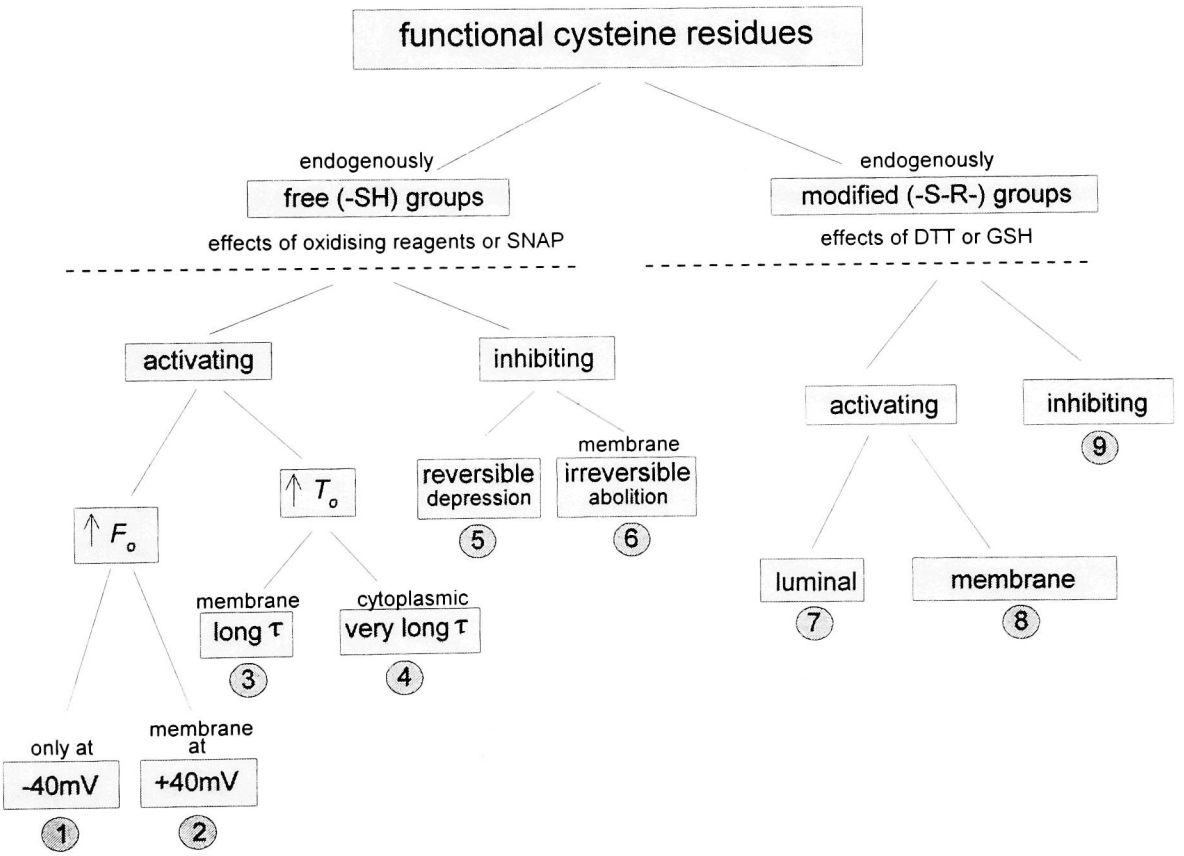

FIG. 1. A chart showing different actions of oxidizing and reducing agents on RyRs. Cysteine residues in proteins are shown as containing either free –SH groups or modified thiols (–S-R–), where R is S in disulfides or N in nitrosothiols. The numbers 1–9 indicate functional subdivisons on the basis of channel activity or accessibility to redox reagents. Effects have been localized to membrane-associated (membrane), cytoplasmic, or luminal domains where indicated. All results were obtained from RyRs incorporated into lipid bilayers from SR vesicles (Laver *et al.*, 1995), and recorded either at *cis* potentials of +40 mV and −40 mV using symmetrical solutions containing, in m*M*: 230 Cs methane sulfonate ($CsCH_3SO_3$), 20 CsCl, 10 *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) and 1 $CaCl_2$ adjusted to a pH of 7.4 with CsOH; or only at +40 mV (using a *cis* solution as above and a *trans* solution containing, in m*M*: 30 $CsCH_3SO_3$, 20 CsCl, 0.1 $CaCl_2$, and 10 TES and was adjusted to a pH of 7.4 with CsOH.

The activating and inhibiting –SH, and –S-R–, groups are divided into subclasses on the basis of changes in single-channel activity upon modification, and accessibility to cytoplasmic or luminal solutions. We have no evidence for the number of thiols in any "class." A starting hypothesis is that a subclass contains one residue per subunit (or two if disulfides are formed), because it is conceptually simpler to imagine that modification of one thiol has a discrete action on RyR activity.

SUBCLASSES OF THIOL GROUPS BASED ON SINGLE-CHANNEL CHARACTERISTICS: FOUR ACTIVATING RESPONSES

Activated RyRs exhibit a higher than normal mean current (I') and open probability (P_o), either because the frequency of opening (F_o) and/or the open time (T_o) increase (where I' is the average of all data points in a segment of channel recording, usually 30 to 60 sec). We postulate that modification of one subclass of –SH increases F_o, whereas modification of a second subclass increases T_o, on the basis of the fact that 4,4'-DTDP, DTNB, and thimerosal preferentially prolonged T_o, whereas low [SNAP] and GTX increased F_o alone (Table 1). In addition, NO (~40 n*M*) (Simonsen *et al.*, 1999) donated by 10 μM SNAP increases F_o, while ~4 μM NO, donated by 1 m*M* SNAP, lengthened T_o (Table 1).

F_o increased in two ways—the GTX-induced increase in F_o was seen at +40 mV (*cis* potential), NO-induced increase in F_o was seen at −40 mV, but *not* +40 mV, suggesting that one class of thiol influences activity at +40 mV and the other at −40 mV. These effects were independent of the bilayer potential when the reagents were added. Two discrete changes were also seen with prolongation of T_o because *cis* 4,4'-DTDP introduced intermediate (τ = 8–10 msec), long (τ = 20–30 msec), and very long (τ = 100 msec) components into the open times of cardiac RyRs, whereas *trans* 4,4'-DTDP and thimerosal (*cis* or *trans*) introduced only intermediate and long components (Table 1; control openings were in one τ of ~1.5 msec). The intermediate/long components may have depended on oxidation of one class of thiols accessible to 4,4'-DTDP and thimerosal, whereas the very long time constants required oxidation of other thiols accessible only to *cis* 4,4'-DTDP. Curiously, only one additional (long) component was added to skeletal RyRs by either *cis* 4,4'-DTDP or *cis* or *trans* DTNB (Table 1; control activity had short and intermediate components). The cysteines supporting the very long component are either absent in skeletal RyRs or are inaccessible to 4,4'-DTDP.

TWO INHIBITING RESPONSES

Inhibition following –SH modification varies between cardiac and skeletal RyRs and between modifying agents. Inhibition by 4,4'-DTDP or thimerosal abolished RyR activity and could not be reversed either by agonists (Ca^{2+} or ATP), or by voltage pulses (which normally activate channels; Laver *et al.*, 1995) (Table 1). Inhibition was prevented if DTT was added before activity was lost, and inhibiton could be reversed by DTT in skeletal, but not cardiac, RyRs (Table 1). Either access to the inhibition site by DTT is denied in cardiac RyRs or the inhibiting cysteines differ between the RyR isoforms. Two different types of inhibition were seen in skeletal RyRs, because 1 m*M* SNAP depressed (but did not abolish) activity, and SNAP-inhibited channels could be reactivated by voltage pulses. The SNAP-induced inhibition may be an enhancement of the usual reduction in channel activity after a voltage pulse (Laver and Lamb, 1998; Zahradnikova and Mezaros, 1998; Haarmann *et al.*, 1999). The reactivation of SNAP-inhibited channels by voltage pulses could then occur via the normal voltage activation of low-activity channels. Modification of one class of thiol might reversibly depress channels, whereas oxidation of a second class irreversibly abolishes activity (Fig. 1).

To reiterate, single-channel analysis shows six different responses to agents that covalently modify thiol groups. This could provide evidence for as many as six classes of thiols, four that activate channels when modified and two that inhibit.

THREE RESPONSES OF ENDOGENOUSLY MODIFIED THIOLS

DTT reduces disulfides and can remove modifying groups from thiols (Xu *et al.*, 1998). Reducing reagents, in the absence of added oxidants, inhibit (Eager *et al.*, 1997; Zable *et al.*, 1997; Marengo *et al.*, 1998) or activate RyRs (Oba *et al.*, 1996b). We found that 2 m*M* DTT in the *trans*, but not *cis*, solution activated rabbit skeletal RyRs and that 10 m*M* DTT on either side of pig skeletal RyRs increased activity, with reversal by 1 m*M* DTNB from either side (Table 2). Activation by DTT was not related to preparative techniques because preparation of pig skeletal SR was based on Meissner (1984) and Ma *et al.* (1995) with buffers containing 2m*M* DTT, whereas preparation of skeletal SR was based on Saito *et al.* (1984), with buffers lacking DTT. Xu *et al.* (1998) also show a higher P_o after adding 10 m*M* DTT to RyRs that were previously activated by CysNO. The results suggest three classes of modified thiols, one enhances activity (its reduction inhibits), and two depress (their reduction activates) (Fig. 1). If each class contains disulfides, at least six cysteine residues per subunit could be involved.

LOCATION OF CYSTEINE RESIDUES

Functional cysteine residues need not be near the ion pore because RyR activity can be regulated via long-range allosteric effects from regions >10 nm from the pore (Wagenknecht *et al.*, 1997), and may be located on associated proteins, such as FKBP12, triadin, or calmodulin (Liu and Pessah, 1994). Lipid-soluble (4,4′-DTDP, NO, or GTX) and water-soluble (thimerosal, DTNB, and DTT) reagents partition into the bilayer (see below) and can modify cysteines in membrane-associated regions of the RyR, as well as on the side to which they are added and on the opposite side, if sufficient time is allowed for the concentration of reagent in the opposite solution to increase to active levels. Addition of reagents to different sides of bilayers has helped locate some classes of cysteines.

ACTIVATING THIOLS IN CYSTOPLASMIC AND MEMBRANE-ASSOCIATED DOMAINS

As mentioned above, *cis* 4,4′-DTDP added intermediate, long, and very long components to open time distributions of cardiac RyRs, whereas *trans* 4,4′-DTDP added only intermediate and long components. This suggests that the very long component depends on oxidation of thiols on the cytoplasmic domain. Thimerosal and 4,4′-DTDP on either side of the bilayer added "intermediate" and "long" time constants, suggesting that thiols responsible for these components were located in membrane-associated domains (the transmembrane region or in regions very close to the membrane; Fig. 1).

DTNB (1 m*M*) on either side of skeletal RyRs introduced one longer time constant component to the open times (Haarmann *et al.*, 1999). Addition of 10 m*M* DTT to the solution containing DTNB (either *cis* or *trans*), or to the opposite solution (either *cis* DTNB followed by *trans* DTT, or *trans* DTNB followed by *cis* DTT) returned activity to control levels. The rate of change in activity was independent of the side of the bilayer that reagents were added to, again suggesting that activating residues were in membrane-associated domains (Fig. 1). Accessibility to water-soluble reagents could suggest a location in the channel pore. However, DTT with a pKa of 9–10 (Shaked *et al.*, 1980) would rapidly partition into the membrane, as would a fraction of DTNB with a pKa of 5–6 (Houk *et al.*, 1987). Thus, these agents could also access transmembrane residues or residues close to the membrane but not in the pore. The agents could not have targetted residues on the opposite side of the channel remote from the membrane because massive dilution in the opposite solution would mean that activation with DTNB or recovery with DTT would have been much slower when reagents were applied to the opposite solution. Differential rates of the effects of the reagents were not observed in skeletal RyRs.

The F_o-enhancing thiols oxidized by GTX are also located in or near the membrane, because GTX increased activity at the same rate after

addition to either *cis* or *trans* solution. Activation by *cis* GTX was reversed by 2 m*M cis* DTT, whereas addition of GTX to the *trans* chamber did not activate RyRs if the *cis* solution contained DTT.

INHIBITING THIOL GROUPS ARE IN THE MEMBRANE DOMAIN

Inhibition of cardiac RyRs was seen within 5 min of adding 4,4′-DTDP or thimerosal to either side of the bilayer (Eager and Dulhunty, 1999), suggesting a membrane-associated site of action. Inhibition of skeletal RyRs by *cis* 4,4′-DTDP is consistent with a membrane-associated action (Haarmann *et al.*, 1999).

ENDOGENOUSLY MODIFIED THIOL GROUPS ARE IN CYTOPLASMIC, MEMBRANE-ASSOCIATED, OR LUMINAL DOMAINS

Cardiac RyRs were inhibited by DTT applied to the *cis* chamber, indicating a cytoplasmic or membrane location for the modified thiols. Skeletal RyRs were activated by 10 m*M* DTT in the *cis* or *trans* chamber, indicating a membrane-associated action. Skeletal RyRs were also activated by 2 m*M* DTT in the *trans* chamber, but not the *cis* chamber, suggesting a modified thiol that is uniquely located on the luminal domain (Fig. 1).

DISCUSSION

RyRs respond to modification, or demodification (with DTT) –SH groups, in nine ways, suggesting that nine classes of cysteines and a minimum of nine residues per subunit can alter RyR gating. This number could double if intraprotein disulfides were involved. If it were argued that different responses reflect different conformations of mixed disulfides or nitrosylated thiols, six classes of cysteines remain: activating cytoplasmic and activating and inhibiting membrane-associated thiols, and endogenously modified thiols that, when reduced, either inhibit or activate. Activating residues are located in both domains of the RyR associated with the membrane and in the luminal domain.

There are several reports of multiple functional sulfhydryls on RyRs. Three classes of –SH explain actions of the alkylating agent, *N*-ethylmaleimide (NEM), the cross-linking reagent, diamine, and NO (Aghdasi *et al.*, 1997a,b). Cross-linking residues were in the central and carboxy-terminal transmembrane part of the RyR, whereas amino-terminal thiols were alkylated by NEM (Wu *et al.*, 1997). Two functional classes explain activation by *S*-nitrosylation of three thiols per subunit (reversed by DTT), no effect of oxidation of another five to six, and activation by oxidation of an additional thiol, which was not reversed by DTT (Xu *et al.*, 1998). A unique decrease in single-channel conductance was seen when methanethiosulfonate derivatives were added to the *cis* side of RyRs (Quinn and Ehrlich, 1997). The relationship between the classes of cysteine residues defined in different laboratories is yet to be determined. It is not clear why some disulfides are reduced by DTT (Wu *et al.*, 1997) whereas others are not (Xu *et al.*, 1998). Because all activating responses in Table 1 were reversed by DTT, the cysteines may have belonged to classes described by Wu *et al.* (1997). Alternatively, reversibility might indicate that mixed disulfides or *S*-nitrosothiols, rather than disulfides, were formed (Xu *et al.*, 1998). More functional cysteines may be discovered as different modifying agents are used under different conditions. The next challenges will be determining which residues in the RyR sequence form each of the functional classes, and the structural basis for their effects on channel gating.

We conclude that between six and nine classes of cysteines can influence RyR activity and provide a basis for *in vivo* modulation of Ca^{2+} release from the SR. Concentrations of oxidants including NO and reducing regents in muscle vary with activity, fatigue, and pathology. A battery of free and modified thiols, with different actions on the RyR channel, allow for a sophisticated differential response to changes in the redox state of the cytoplasm and the functional state of the muscle.

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ABBREVIATIONS

ATP, adenosine triphosphate; Ca^{2+}, calcium ion; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); 4,4′-DTDT, 4,4′-Dithiodipyridine; DTT, dithiothreitol; F_o, frequency of opening; GTX, gliotoxin; I', mean current (mean amplitude of all data points in a period of recording, including channel openings and closures); NEM, *N*-ethylmaleimide; NO, nitric oxide; P_o, open probability; RyR, ryanodine receptor; –SH, sulfhydryl; SNAP, *S*-nitroso-*N*-acetyl-penicillamine; SR, sarcoplasmic reticulum; T_o, mean open time; τ, exponential time constant.

REFERENCES

ABRAMSON, J.J., and SALAMA, G. (1989). Critical sulfhydryls regulate calcium release from sarcoplasmic reticulum. J. Bioenerg. Biomembr. **21,** 283–294.

AGHDASI, B., REID, M.B., and HAMILTON, S.L. (1997a). Nitric oxide protects the skeletal muscle Ca^{2+} release channel from oxidation induced activation. J. Biol. Chem. **272,** 25462–25467.

AGHDASI, B., ZHANG, J.Z., WU, Y.L., REID, M.B., and HAMILTON, S.L. (1997b). Multiple classes of sulfhydryls modulate the skeletal muscle Ca^{2+} release channel. J. Biol. Chem. **272,** 3739–3748.

DULHUNTY, A.F., JUNANKAR, P.R., EAGER, K.R., AHERN, G.P., and LAVER, D.R. (1996). Ion channels in the sarcoplasmic reticulum of striated muscle. Acta Physiol. Scand. **156,** 375–385.

EAGER, K.R., and DULHUNTY, A.F. (1998). Activation of the cardiac ryanodine receptor by sulfhydryl oxidation is modified by Mg^{2+} and ATP. J. Membr. Biol. **163,** 9–18.

EAGER, K.R., and DULHUNTY, A.F. (1999). Cardiac ryanodine receptor activity is altered by oxidizing reagents in either the luminal or cytoplasmic solution. J. Membr. Biol. **167,** 205–214.

EAGER, K.R., RODEN, L.D., and DULHUNTY, A.F. (1996). Actions of sulfhydryl reagents on single ryanodine receptor Ca^{2+}-release channels from sheep myocardium. Am. J. Physiol. **272,** C1908–C1918.

GUSE, A.H., DA SILVA, C.P., BERG, I., SKAPENKO, A.L., WEBER, K., HEYER, P., HOHENEGGER, M., ASHAMUS, G.A., SCHULZE-KOOPS, H., POTTER, V.L.P., and MAYR, G.W. (1999). Regulation of calcium signalling in T lymphocytes by the second messenger cyclic ADP-ribose. Nature **398,** 70–73.

HAARMANN, C.S., FINK, R.A.H., and DULHUNTY, A.F. (1999). Oxidation and reduction of pig skeletal muscle ryanodine receptors. Biophys. J. **77,** 3010–3022.

HART, J.D., and DULHUNTY, A.F. (1999). Nitric oxide activates or inhibits skeletal muscle ryanodine receptors depending on its concentration, membrane potential and ligand binding. J. Membr. Biol. (in press).

HOUK, J., SINGH, R., and WHITESIDES, G.M. (1987). Measurement of thiol-disulfide interchange reactions and thiol pKa values. Methods Enzymol. **143,** 129–140.

LAVER, D.R., and LAMB, G.D. (1998). Inactivation of Ca^{2+} release channels (ryanodine receptors RyR1 and RyR2) with rapid steps in $[Ca^{2+}]$ and voltage. Biophys. J. **74,** 2352–2364.

LAVER, D.R., RODEN, L.D., AHERN, G.P., EAGER, K.R., JUNANKAR, P.R., and DULHUNTY, A.F. (1995). Cytoplasmic Ca^{2+} inhibits the ryanodine receptor from cardiac muscle. J. Membr. Biol. **147,** 7–22.

LIU, G., and Pessah, I.N. (1994). Molecular interaction between ryanodine receptor and glycoprotein triadin involves redox cycling of functionally important hyperreactive sulfhydryls. J. Biol. Chem. **269,** 33028–33034.

MA, J., BHAT, M.B., and ZHAO, J. (1995). Rectification of skeletal muscle ryanodine receptor by FK506 binding protein. Biophys. J. **69,** 2398–2404.

MARENGO, J.J., HIDALGO, C., and BULL, R. (1998). Sulfhydryl oxidation modifies the calcium dependence of ryanodine-sensitive calcium channels of excitable cells. Biophys. J. **74,** 1263–1277.

MEISSNER, G. (1984). Adenine nucleotide stimulation of Ca^{++}-induced Ca^{++} release in sarcoplasmic reticulum. J. Biol. Chem. **259,** 2365–2374.

OBA, T., KOSHITA, M., and YAMAGUCHI, M. (1996a). H_2O_2 modulates twitch tension and increases P_o of Ca^{++} release channel in frog skeletal muscle. Am. J Physiol. **271,** C810–C818.

OBA, T., NIHONYANAGI, K., TANGKAWATTANA, P., and YAMAGUCHI, M. (1996b). A novel phasic contraction induced by dithiothreitol in frog skeletal muscle. Gen. Pharmacol. **27,** 1361–1366.

QUINN, K.E., and EHRLICH, B.E. (1997). Methanethiosulfonate derivatives inhibit current through the ryanodine receptor/channel. J. Gen. Physiol. **109,** 255–264.

REID, M.B. (1998). Role of nitric oxide in skeletal muscle: synthesis, distribution and functional importance. Acta. Physiol. Scand. **162,** 401–409.

SAITO, A., SEILER, S., CHU, A., and FLEISCHER, S. (1984). Preparation and morphology of sarcoplasmic reticulum terminal cisternae from rabbit skeletal muscle. J. Cell Biol. **99,** 875–885.

SAYERS, L.G., BROWN, G.R., MICHELL, R.H., and MICHELANGELI, F. (1993). The effects of thimerosal on calcium uptake and inositol 1,4,5-trisphosphate-induced calcium release in cerebellar microsomes. Biochem. J. **289,** 883–887.

SHAKED, Z., SZAJEWSKI, R.P., and WHITESIDES, G.M.

(1980). Rates of thio-disulfide interchange reactions involving proteins and measurements of thiol pKa values. Biochemistry **19,** 4156–4166.

SIMON, D.I., MULLINS, M.E., JIA, L., GASTON, B., SINGEL, D.J., and STAMLER, J.S. (1996). Polynitrosylated proteins: characterization, bioactivity, and functional consequences. Proc. Natl. Acad. Sci. USA **93,** 4736–4741.

SIMONSEN, U., WADSWORTH, R.M., BUUS, N.H., and MULVANY, M.J. (1999). In vitro simultaneous measurements of relaxation and nitric oxide concentration in rat superior mesenteric artery. J. Physiol. **516,** 271–282.

WAGENKNECHT, T., RADERMACHER, M., GRASSUCCI, R., BERKOWITZ, J., XIN, H.B., and FLEISCHER, S. (1997). Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. J. Biol. Chem. **272,** 32463–32471.

WU, Y., AGHDASI, B., DOU, S.J., ZHANG, J.Z., LIU, S.Q., and HAMILTON, S.L. (1997). Functional interactions between cytoplasmic domains of the skeletal muscle Ca^{2+} release channel. J. Biol. Chem. **272,** 25051–25061.

XU, L., EU, J.P., MEISSNER, G., and STAMLER, J.S. (1998). Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. Science **279,** 234–237.

ZABLE, A.C., FAVERO, T.G., and ABRAMSON, J.J. (1997). Glutathione modulates ryanodine receptor from skeletal muscle sarcoplasmic reticulum. Evidence for redox regulation of the Ca^{2+} release mechanism. J. Biol. Chem. **272,** 7069–7077.

ZAHRADNIKOVA, A., and MEZAROS, G.L. (1998). Voltage change-induced gating transitions of the rabbit skeletal muscle Ca^{2+} release channel. J. Physiol. **509,** 29–38.

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2. Angela F. Dulhunty, Ruwani Hewawasam, Dan Liu, Marco G. Casarotto, Philip G. Board. 2011. Regulation of the cardiac muscle ryanodine receptor by glutathione transferases. *Drug Metabolism Reviews* **43**:2, 236-252. [CrossRef]
3. Lufang Zhou, Miguel A. Aon, Ting Liu, Brian O'Rourke. 2011. Dynamic modulation of Ca2+ sparks by mitochondrial oscillations in isolated guinea pig cardiomyocytes under oxidative stress. *Journal of Molecular and Cellular Cardiology* . [CrossRef]
4. Suresh K. JosephRole of Thiols in the Structure and Function of Inositol Trisphosphate Receptors **66**, 299-322. [CrossRef]
5. V. L. Caulfield, C. Balmer, L. J. Dawson, P. M. Smith. 2009. A role for nitric oxide-mediated glandular hypofunction in a non-apoptotic model for Sjogren's syndrome. *Rheumatology* **48**:7, 727-733. [CrossRef]
6. Chris Jalilian , Esther M. Gallant , Philip G. Board , Angela F. Dulhunty . 2008. Redox Potential and the Response of Cardiac Ryanodine Receptors to CLIC-2, a Member of the Glutathione S-Transferase Structural Family. *Antioxidants & Redox Signaling* **10**:10, 1675-1686. [Abstract] [Full Text PDF] [Full Text PDF with Links]
7. L HOOL. 2007. What Cardiologists Should Know About Calcium Ion Channels and Their Regulation by Reactive Oxygen Species. *Heart, Lung and Circulation* **16**:5, 361-372. [CrossRef]
8. Dr. Livia C. Hool , Ben Corry . 2007. Redox Control of Calcium Channels: From Mechanisms to Therapeutic Opportunities. *Antioxidants & Redox Signaling* **9**:4, 409-435. [Abstract] [Full Text PDF] [Full Text PDF with Links]
9. Benjamin S. Marinov , Rotimi O. Olojo , Ruohong Xia , Jonathan J. Abramson . 2007. Non-Thiol Reagents Regulate Ryanodine Receptor Function by Redox Interactions That Modify Reactive Thiols. *Antioxidants & Redox Signaling* **9**:5, 609-621. [Abstract] [Full Text PDF] [Full Text PDF with Links]
10. H CHANG, C LIU, C CHOU, C HSIEH, C CHANG, W CHEN, S LIU, S HSU, J CHEN, B JIANN. 2005. Thimerosal-induced cytosolic Ca elevation and subsequent cell death in human osteosarcoma cells. *Pharmacological Research* **52**:4, 328-333. [CrossRef]
11. Angela F Dulhunty, Pierre Pouliquin. 2003. What we don't know about the structure of ryanodine receptor calcium release channels. *Clinical and Experimental Pharmacology and Physiology* **30**:10, 713-723. [CrossRef]
12. E Weir. 2002. Redox signaling in oxygen sensing by vessels. *Respiratory Physiology & Neurobiology* **132**:1, 121-130. [CrossRef]
13. Angela F. Dulhunty, Derek Laver, Suzanne M. Curtis, Suzi Pace, Claudia Haarmann, Esther M. Gallant. 2001. Characteristics of Irreversible ATP Activation Suggest that Native Skeletal Ryanodine Receptors Can Be Phosphorylated via an Endogenous CaMKII. *Biophysical Journal* **81**:6, 3240-3252. [CrossRef]