*Journal of Bioenergetics and Biomembranes, Vol. 21, No. 2, 1989*

# MINI-REVIEW

# Critical Sulfhydryls Regulate Calcium Release from Sarcoplasmic Reticulum

Jonathan J. Abramson[1] and Guy Salama[2]

*Received June 30, 1988*


## Abstract

Rapid $Ca^{2+}$ release from the sarcoplasmic reticulum (SR)[3] can be triggered by either binding of heavy metals to a sulfhydryl (SH) group or by catalyzing the oxidation of endogenous groups to a disulfide. $Ca^{2+}$ release has been monitored directly using isolated vesicle preparations or indirectly by monitoring phasic contractions in a skinned fiber preparation. SH oxidation triggered by addition of $Cu^{2+}$/mercaptans, phthalocyanine dyes, reactive disulfides, and various anthraquinones appears to involve a direct interaction with the $Ca^{2+}$ release protein from the SR. A model is presented in which reversible oxidation and reduction of endogenous SH groups results in the opening and closing of the $Ca^{2+}$ release channel from the SR.


**Key Words:**Sulfhydryls; calcium release; sarcoplasmic reticulum; phasic contractions; mercaptans; phthalocyanine dyes; reactive disulfides; anthraquinones.

## Introduction

In spite of recent advances in identification of the $Ca^{2+}$-release protein from sarcoplasmic reticulum (SR), the molecular mechanism by which $Ca^{2+}$ is released from the SR remains unknown (Imagawa *et al.*, 1987; Lai *et al.*, 1988; Hymel *et al.*, 1988). Several theories have been proposed to explain the nature of the coupling between depolarization of the transverse (T)-tubule membrane and release of $Ca^{2+}$ from the SR (Ford and Podolsky, 1972;

[1]Physics Department, Environmental Sciences and Resources Program, Portland State University, Portland, Oregon 97207.
[2]Department of Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261.
[3]Abbreviations: SR, sarcoplasmic reticulum; SH sulfhydryl; T-tubule, transverse tubule; 2,2′-DTDP, 2,2′-dithiodipyridine; 4,4′-DTDP, 4,4′-dithiodipyridine; DTT, dithiothreitol.



0145-479X/89/0400-0283$06.00/0 

Fabiato and Fabiato, 1977; Endo *et al.*, 1970; Meissner, 1984; Schneider and Chandler, 1973). A substantial amount of evidence has been presented indicating that the release mechanism is distinct from the $Ca^{2+}$ uptake pathway (Imagawa *et al.*, 1987; Lai *et al.*, 1988; Hymel *et al.*, 1988; Inui *et al.*, 1987). However, little is known regarding the chemical interaction that causes the opening and closing of the $Ca^{2+}$ release channel. In this article, we describe how interactions with sulfhydryl (SH) groups, on or associated with the $Ca^{2+}$ release channel, affect the state of the release pathway. Binding of heavy metals or oxidation of an endogenous SH group causes the $Ca^{2+}$ permeability of the SR to increase dramatically. Reduction of the disulfide formed causes the $Ca^{2+}$ permeability to decrease and the SR to return to its initial state. Similar observations have been made with both isolated SR vesicles and with chemically skinned muscle fibers.

## Results and Discussion

### *Interaction with Heavy Metals*

Our interest in SH groups involved in $Ca^{2+}$ release from the SR started with the observation that micromolar concentrations of heavy metals (e.g., $Hg^{2+}$, $Ag^{+}$, $Cu^{2+}$ and $Cd^{2+}$) induce rapid $Ca^{2+}$ release from SR vesicles (Abramson *et al.*, 1983). By preventing the development of a large $Ca^{2+}$ gradient across the SR, these metals are also observed to stimulate $Ca^{2+}$-ATPase activity with actively loaded SR vesicles. This apparent uncoupling of ATPase activity from $Ca^{2+}$ uptake is caused by $Hg^{2+}$ stimulating the $Ca^{2+}$ release activity of the SR. It is not due to an interaction with the $Ca^{2+}$ pump, as previously suggested (Shamoo and MacLennan, 1975).

The first indication that a SH group was important in the $Ca^{2+}$ release mechanism came from determining the relative effectiveness of the different heavy metals in stimulating $Ca^{2+}$ release from SR vesicles. We observed that those metals that were most effective in stimulating $Ca^{2+}$ release from SR vesicles were those that bound with highest affinity to SH-containing reagents (Abramson *et al.*, 1983). This SH group that binds heavy metals and causes a large increase in the SR $Ca^{2+}$ permeability was also found to be in a relatively hydrophilic environment (Abramson *et al.*, 1983). SH reagents such as Salyrgan, *N*-ethylmaleimide, and other organic mercurials induce $Ca^{2+}$ release at slower rates than such probes as $Hg^{2+}$ and $Ag^{+}$ (Martonosi and Feretos, 1964; Abramson *et al.*, 1983; Bindoli and Fleischer, 1983).

At this point, it was important to determine whether heavy-metal-induced $Ca^{2+}$ release was a specific interaction with the $Ca^{2+}$ release protein or a nonspecific interaction with other components of the SR membrane.

Known inhibitors of $Ca^{2+}$ release such as ruthenium red (micromolar), tetracaine (submillimolar), procaine and $Mg^{2+}$ (millimolar) were shown to inhibit $Ag^{+}$-induced $Ca^{2+}$ release (Salama and Abramson, 1984). Adenine-containing nucleotides, which had been shown to stimulate $Ca^{2+}$ release in a $Ca^{2+}$-dependent manner, was also found to stimulate $Ag^{+}$-induced $Ca^{2+}$ release. Most significantly, those adenine-containing nucleotides that were most potent stimulators of $Ca^{2+}$-induced $Ca^{2+}$ release (Meissner, 1984; Meissner *et al.*, 1986) were also found to be most effective in enhancing $Ag^{+}$-induced $Ca^{2+}$ release (Stuart and Abramson, 1988). We also observed that $Ca^{2+}$ release induced by $Ag^{+}$ is considerably faster when assayed with SR vesicles derived from the terminal cisternae region (HSR) than from SR derived from the longitudinal region (LSR). It appears as if heavy metals trigger $Ca^{2+}$ release through the same protein or proteins that are responsible for $Ca^{2+}$-induced $Ca^{2+}$ release. Interestingly, $Ag^{+}$-induced $Ca^{2+}$ release is optimal under physiological conditions, at pH 7.0, and at 1 mM free $Mg^{2+}$ (Salama and Abramson, 1984). This is the only way that $Ca^{2+}$-induced and $Ag^{+}$-induced $Ca^{2+}$ releases differ. $Ca^{2+}$-induced release is further stimulated as the extravesicular $Mg^{2+}$ concentration decreases to zero $Mg^{2+}$.

It is highly unlikely that heavy metals play any part in excitation–contraction coupling. What is more significant than the specific reagent used to induce $Ca^{2+}$ release is the binding site, the SH group, with which the heavy metals interact. A large number of proteins contain SH groups that undergo oxidation–reduction reactions on a millisecond time scale (Williams, 1976). The $Ca^{2+}$ permeability of human platelets (Adunyah and Dean, 1986), insulinoma cells (Erlichman *et al.*, 1979), liver microsomes (Thor *et al.*, 1985), and mitochondria (Beatrice *et al.*, 1984) have all been shown to be regulated by SH interactions. Both extracellular $Ca^{2+}$ and oxidation increase acetylcholine release from the frog muscle endplate (Kosower and Werman, 1971). SH group oxidation–reduction may play a general role in controlling the state of many membrane-bound transport proteins. More physiologically significant than the interaction with heavy metals was our observation that rapid $Ca^{2+}$ release can be induced in both isolated vesicles and in skinned muscle fibers by oxidation reactions (Trimm *et al.*, 1986; Pike *et al.*, 1987; Abramson, *et al.*, 1988*b*).

### *Sulfhydryl Oxidation*

Copper is known to catalyze the oxidation of cysteine to cystine. We have observed that catalytic concentrations of $Cu^{2+}$ (2–5 $\mu$M), in the presence of micromolar concentrations of various mercaptans, induce rapid $Ca^{2+}$ release from either actively or passively loaded SR vesicles (Trimm *et al.*, 1986). At these concentrations, $Cu^{2+}$ alone, mercaptan alone, or a premixed

solution of $Cu^{2+}$ plus mercaptan were ineffective in stimulating $Ca^{2+}$ release. Oxidized mercaptans such as cystine and cystamine were also not at all effective in causing $Ca^{2+}$ release from SR vesicles. However, sequential additions of $Cu^{2+}$ and mercaptan were very effective in stimulating release of $Ca^{2+}$. We proposed that release was triggered by a $Cu^{2+}$-catalyzed oxidation of added cysteine to an endogenous critical SH group on or near the $Ca^{2+}$-release site. Evidence supporting an oxidation reaction is as follows:

1. The rate of $Cu^{2+}$-catalyzed oxidation of the various mercaptans tested paralleled the rate of $Ca^{2+}$ release induced by these mercaptans.
2. Addition of the reducing agent dithiothreitol (DTT) reverses the $Cu^{2+}$/cysteine effect. The $Ca^{2+}$ channel closes and $Ca^{2+}$ is actively reaccumulated by the SR.
3. The $Ca^{2+}$ release rate induced by $Cu^{2+}$/cysteine was first order with respect to $Cu^{2+}$, as expected for a $Cu^{2+}$-catalyzed oxidation reaction (Trimm *et al.*, 1986).
4. Using [$^{35}$S]cysteine plus $Cu^{2+}$, we have observed covalent labeling of several SR proteins, which is not seen in the absence of added $Cu^{2+}$ (unpublished data).

As in the case of heavy-metal-stimulated $Ca^{2+}$ release, $Cu^{2+}$/mercaptan-induced $Ca^{2+}$ release was inhibited by ruthenium red, tetracaine, procaine, and $Mg^{2+}$ (Trimm *et al.*, 1986) and was enhanced by adenine nucleotides and caffeine (Stuart and Abramson, 1988). $Ca^{2+}$ release was also optimal at physiological pH, and the $Ca^{2+}$ release rate increased when the $Mg^{2+}$ concentration was reduced to $< 1$ mM. The oxidation reaction caused by the addition of $Cu^{2+}$ and cysteine appears to open the $Ca^{2+}$ release channel from the terminal cisternae region of the SR. Subsequent reduction of the disulfide formed causes the $Ca^{2+}$ transport pathway to close and the released $Ca^{2+}$ to be actively reaccumulated by the SR vesicles.

Despite the evidence presented above, it is difficult to prove conclusively that the combination of $Cu^{2+}$ and cysteine oxidizes free SH groups to form a mixed disulfide with an SR protein. Reactive disulfide reagents such as 2,2′-dithiodipyridine (2,2′-DTDP) and 4,4′-dithiodipyridine (4,4′-DTDP) are known to oxidize SH groups without the addition of a catalyst such as $Cu^{2+}$. This class of compounds is absolutely specific for free SH groups. They act at low substrate concentrations, and the oxidation reaction can be quantitatively monitored by spectrophotometric measurements of thiopyridone production. These reagents also possess an important property that enables them to oxidize SH groups of low pKa selectively (Brocklehurst, 1979). The addition of 2,2′-DTDP (10–20 $\mu$M) or 4,4′-DTDP (20–30 $\mu$M) to $Ca^{2+}$-loaded SR vesicles results in a rapid release of $Ca^{2+}$ and thiopyridone into the

medium (Salama *et al.*, 1988). Subsequent addition of glutathione (0.5 mM) or DTT (1.0 mM) reduced the disulfide bond and initiated the rapid reuptake of the released $Ca^{2+}$. Release induced by 2,2′-DTDP was optimal at 1 mM free $Mg^{2+}$, and was inhibited by millimolar concentrations of adenine nucleotides. These compounds offer a new and powerful approach to tag the $Ca^{2+}$ release channel with various covalent labels and thereby probe possible allosteric sites of agents that modulate $Ca^{2+}$ release (Salama *et al.*, 1988; Zaidi *et al.*, 1988).

Calcium release induced by heavy metals (Aoki *et al.*, 1986; Pike *et al.*, 1987), by SH oxidation (Salama *et al.*, 1986), and by reactive disulfide reagents (Salama *et al.*, 1988) has also been observed by monitoring isometric tension in skinned rabbit psoas fibers. Low concentrations of heavy metals (5–50 $\mu$M) induced contractions with a rapid onset of tension generation followed by a relaxation phase. Higher concentrations of heavy metals produced rapid tonic tension as the SR network became irreversibly permeable to $Ca^{2+}$. The presence of micromolar concentrations of $Cu^{2+}$ followed by the addition of cysteine elicited phasic contractions that were blocked by SH-reducing agents (i.e., 5 mM DTT). As in the case of vesicle experiments, contractions are blocked by micromolar concentrations of ruthenium red and millimolar concentrations of $Mg^{2+}$ (Pike *et al.*, 1987). These results show that this critical SH site at the triadic junction is readily accessible from the cytosolic milieu, as well as in isolated terminal cisternae vesicles.

### *Phthalocyanine Dyes*

Many enzymes known to catalyze biological redox reactions contain iron or copper redox centers. Phthalocyanine dyes are structurally similar to metalloporphyrins, which are known to be involved in biological redox reactions (i.e., cytochromes, catalase, and peroxidase). In recent studies, we have shown that several phthalocyanine dyes, most notably alcian blue, are very potent stimulators of $Ca^{2+}$ release from SR vesicles (Abramson *et al.*, 1988*b*). At an alcian blue concentration of 5 $\mu$M and a free $Mg^{2+}$ concentration of 1 mM, we measured $Ca^{2+}$ efflux rates of $> 20$ nmol/mg/sec. Unlike $Ca^{2+}$-induced $Ca^{2+}$ release, which is relatively slow at a physiological free-$Mg^{2+}$ concentration of $\sim 1$ mM, most of the SH reagents discussed here, especially alcian blue, are extremely potent $Ca^{2+}$-releasing agents even at 1 mM $Mg^{2+}$. The phthalocyanine dyes, like all of the other SH-reactive compounds, appears to interact directly with the $Ca^{2+}$ release mechanism of the SR. Release is inhibited by ruthenium red, tetracaine, and $Mg^{2+}$. It is stimulated by the presence of adenine nucleotides and is specific for terminal cisternae SR.

The $Cu^{2+}$ phthalocyanine dye alcian blue also appears to induce $Ca^{2+}$ release via an oxidation reaction. Reduction of alcian blue by the addition of

$Na_2S_2O_4$, sodium dithionite, results in a change in the dye spectrum and a total inhibition of the $Ca^{2+}$ release activity of the dye. Reoxidation of alcian blue by molecular $O_2$ shifts the spectrum back to the untreated state, and restores the $Ca^{2+}$ release activity of the dye. Products of dithionite oxidation are not responsible for inhibition of release activity since their presence in the reaction medium does not inhibit $Ca^{2+}$ release activity of reoxidized alcian blue. Pretreatment of alcian blue with a large excess of DTT or cysteine also reduces alcian blue and prevents activation of $Ca^{2+}$ release. If, instead of preincubating alcian blue with reducing agent, DTT is added upon the completion of alcian-blue-induced $Ca^{2+}$ release, $\sim 80\%$ of the released $Ca^{2+}$ is actively reaccumulated by the SR. Reversal of alcian-blue-induced $Ca^{2+}$ release by addition of DTT is not caused by an interaction with the $Ca^{2+}$ pump from the SR, but by an interaction with the $Ca^{2+}$ release pathway. In principle, alcian blue can oxidize SH groups in several ways. However, given the observation that DTT closes the $Ca^{2+}$ release pathway opened by alcian blue, it is likely that the primary mode of action of the phthalocyanine dyes is to oxidize two neighboring SH groups associated with the $Ca^{2+}$ release mechanism. Unlike the $Cu^{2+}$/mercaptan oxidation reaction that involves the formation of mixed disulfides, in which only one endogenous SR SH group is involved, alcian-blue-stimulated $Ca^{2+}$ release appears to involve the oxidation of two neighboring SH groups (Abramson *et al.*, 1988*b*). Previous studies with cupric phenanthroline, which is known to catalyze the air oxidation of SH groups to disulfides, also reported an increase in the $Ca^{2+}$ permeability of the SR membrane (Abramson *et al.*, 1983).

### *Anthraquinones*

Quinones, which are widely distributed in higher plants and throughout the animal kingdom, are known to play a central role in many electron-transport systems such as cellular respiration and photosynthesis (Crane, 1968). Anthraquinones are also of great clinical interest because they are widely used antineoplastic agents. Unfortunately their use is limited by several side effects, the most serious of which is chronic cardiotoxicity. The anthraquinone doxorubicin, or Adriamycin, has been shown to be very effective in inducing $Ca^{2+}$ release from SR vesicles and in causing phasic contractions in skinned muscle fibers (Zorzato *et al.*, 1985). Photolabeled [$^{14}$C]doxorubicin has also been used to label several high-molecular-weight proteins (Zorzato *et al.*, 1986), one of which has been recently implicated in the $Ca^{2+}$ release process (Imagawa *et al.*, 1987; Lai *et al.*, 1988; Hymel *et al.*, 1988). To determine whether $Ca^{2+}$ release induced by doxorubicin was an isolated effect, or due to a more general interaction between the $Ca^{2+}$ release mechanism and quinones, we tested the $Ca^{2+}$ release activity of several

related quinones (Abramson *et al.*, 1988*a*). The benzoquinones tested showed no $Ca^{2+}$ release activity. Some of the naphthoquinones, such as plumbagin (Trimm *et al.*, 1986) and menadione, did cause $Ca^{2+}$ release at concentrations of tens of micromolars. Anthraquinones such as doxorubicin and daunorubicin were very active $Ca^{2+}$-releasing agents at $< 10\,\mu M$. The most effective of the anthraquinones tested was mitoxantrone. It induced rapid release in SR vesicles and fast phasic contractions in chemically skinned muscle fibers (Abramson *et al.*, 1988*a*).

Anthraquinone-induced $Ca^{2+}$ release was inhibited by submicromolar ruthenium red and millimolar $Mg^{2+}$. It was stimulated by adenine nucleotides ($\sim$ 10-fold), and strongly activated by submicromolar concentrations of $Ca^{2+}$ (Abramson *et al.*, 1988*a*). [$^3$H]Ryanodine binding to terminal cisternae SR vesicles has been shown to parallel closely the transport characteristics of $Ca^{2+}$-induced $Ca^{2+}$ release (Pessah *et al.*, 1986, 1987). We have observed that doxorubicin both stimulates ryanodine binding and induces a dose-dependent decrease in $K_{d/Ca^{2+}}$. In a similar manner to caffeine (20 mM), doxorubicin (60 $\mu$M) increases the apparent affinity of the $Ca^{2+}$ activator site for $Ca^{2+}$. Further comparisons between the action of doxorubicin and caffeine led to the finding that caffeine both decreases doxorubicin-stimulated high-affinity ryanodine binding to its receptor and inhibits doxorubicin-induced $Ca^{2+}$ release from the SR (Abramson *et al.*, 1988*a*). The interaction between the anthraquinone and caffeine-binding sites is described in more detail in a recent manuscript (Abramson *et al.*, 1988*a*). Doxorubicin and caffeine appear to modify the $Ca^{2+}$ transport properties of the SR by a direct interaction with the $Ca^{2+}$–ryanodine complex, which has recently been identified as the $Ca^{2+}$ release protein from the junctional face membrane of the SR.

Unlike channel activators such as ATP, $Ca^{2+}$, and doxorubicin, which increase [$^3$H]ryanodine binding to its receptor, addition of $Ag^+$ and aryldisulfides causes a rapid displacement of bound ryanodine from its receptor (Pessah *et al.*, 1987). While activation of ryanodine binding is correlated with the $Ca^{2+}$ channel being in the open state, displacement of bound ryanodine caused by the addition of SH reagents appears to be caused by a more direct interaction between the ryanodine-binding site and the critical SH group that we have implicated in the $Ca^{2+}$ release process. If doxorubicin is stimulating $Ca^{2+}$ release and ryanodine binding by interacting with SR SH groups, this is different than the interaction between $Ag^+$ and aryldisulfides that results in the displacement of bound ryanodine from the SR. Doxorubicin-induced $Ca^{2+}$ release is likely to involve the oxidation of two or more neighboring SH groups, while aryldisulfide or $Ag^+$ probably involves an interaction with only one endogenous SH group.

One of the leading hypotheses describing the molecular mechanism of doxorubicin cardiotoxicity involves free-radical damage and nonspecific

oxidation of the cardiac membranes (Doroshow, 1983). To determine whether anthraquinone-induced $Ca^{2+}$ release was caused by superoxide radicals generated during anthraquinone redox cycling, superoxide dismutase and catalase were added to the buffer in which $Ca^{2+}$ release was monitored. Superoxide dismutase catalyzes the aqueous disproportionation of superoxide radical to $H_2O_2$ and molecular oxygen, while catalase catalyzes the aqueous reduction of $H_2O_2$ to $H_2O$. These enzymes had no effect on $Ca^{2+}$ efflux rates induced by $Cu^{2+}$/cysteine (Trimm *et al.*, 1986), phthalocyanine dyes (Abramson *et al.*, 1988*b*), or doxorubicin (Abramson *et al.*, 1988*a*). It is likely that, if oxidative damage to cardiac membranes is caused by doxorubicin's interaction with the SR membrane, then oxidative coupling is due to a direct interaction with the $Ca^{2+}$ release protein from SR.

### *Proposed Model*

The mechanism by which depolarization of the T-tubule is coupled to $Ca^{2+}$ release from the SR is unknown. We have shown that SH oxidation triggers rapid $Ca^{2+}$ release from terminal cisternae SR, and that reduction of the disulfide formed causes the $Ca^{2+}$ release pathway to close (Trimm *et al.*, 1986; Abramson *et al.*, 1988*b*). Furthermore, SH reagents appear to interact with the high-molecular-weight $Ca^{2+}$–ryanodine-binding complex that has recently been identified as the $Ca^{2+}$ release protein. This statement is supported by ryanodine-binding studies (Pessah *et al.*, 1987; Abramson *et al.*, 1988*a*) and our observation that all stimulators and inhibitors of $Ca^{2+}$-induced $Ca^{2+}$ release from SR vesicles also affect SH-induced $Ca^{2+}$ release in an analogous fashion.

Whether or not these SH groups are directly involved in the triggering of "physiological $Ca^{2+}$ release" is not known. It may be that SH interactions do not play an integral part in excitation–contraction coupling, but instead act to regulate the $Ca^{2+}$ release activity over a longer time scale. On the other hand, it is quite possible that SH oxidation and reduction is directly involved in gating of the $Ca^{2+}$ release channel. There is clearly a strong correlation between $Ca^{2+}$-induced and SH-oxidation-induced $Ca^{2+}$ release from SR. We have proposed a testible working model for a SH-controlled gating mechanism for the $Ca^{2+}$ release channel from the SR (Abramson and Salama, 1988). In Fig. 1, we show three SH groups involved in the gating of the $Ca^{2+}$ release channel. So far, our discussion has focused on two neighboring SH groups that, when oxidized to a disulfide, induce rapid $Ca^{2+}$ release from SR vesicles. We have recently observed that increasing the $Ag^+$ concentration to $> 10\,\mu M$ results in partial inhibition of $Ag^+$-stimulated $Ca^{2+}$ release from SR (Moutin *et al.*, 1988). There appears to be a third SH group ($SH_3$), which may be involved in closing down the $Ca^{2+}$ release channel.

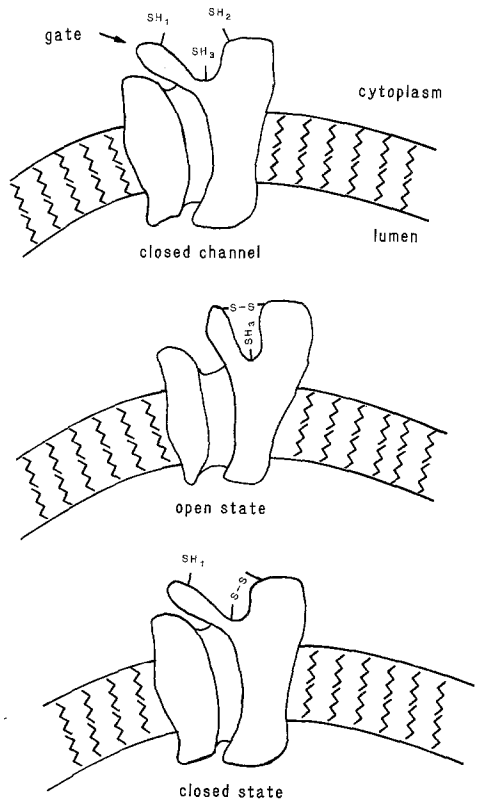


**Fig. 1.** Model of the $Ca^{2+}$ release protein gated by SH oxidation and reduction of three endogenous SH groups. From Abramson and Salama, *Mol. Cell. Biochem.* (1988). **82**, 81–84; reprinted with permission; Martinus Nijhoff Publishers, Boston.

In Fig. 1, $SH_1$ is the reactive critical SH group that, when oxidized by exogenously added $Cu^{2+}$/cysteine (Trimm *et al.*, 1986) or reactive disulfides (Salama *et al.*, 1988), upon binding of heavy metals (Abramson *et al.*, 1983; Salama and Abramson, 1984), or when cross-linked to $SH_2$, causes the $Ca^{2+}$ release channel to open (Abramson *et al.*, 1988*b*). Shortly after SH oxidation and the formation of the disulfide $S_1$–$S_2$, rapid SH–disulfide interchange occurs, leaving $SH_1$ in the reduced form and $S_2$–$S_3$ oxidized. This rapid exchange reaction causes the closing of the $Ca^{2+}$ release pathway. The channel is now either partially or totally in the closed configuration since $SH_1$ is reduced and $SH_3$ is oxidized. In this state, the closed channel cannot respond to further excitation from the T-tubule. On a slower time scale, a reducing agent, perhaps reduced glutathione, or an endogenous enzyme

catalyzes the reduction of $S_2$–$S_3$, resetting the system to its initial configuration, totally reduced.

The role of $Ca^{2+}$, ATP, $Mg^{2+}$, and other known effectors of $Ca^{2+}$ release might be expected to interface with this model at each of the three steps shown:

1. They might affect the rate of the initial oxidation (the formation of $S_1$–$S_2$). For this step to be physiologically relevant, it must be fast. The oxidation is likely to be catalyzed by an endogenous SR or T-tubule enzyme. The activity of this enzyme, which catalyzes the initial oxidation step that opens the $Ca^{2+}$ release channel, would be stimulated by ATP and micromolar concentrations of $Ca^{2+}$.

2. The rate of SH–disulfide interchange is directly related to the rate at which the channel closes. Activators of $Ca^{2+}$ release such as ATP and $Ca^{2+}$ might prevent or inhibit SH–disulfide interchange, and therefore increase the channel open time. Theories such as $Ca^{2+}$-induced $Ca^{2+}$ release suggest that optimal $Ca^{2+}$ (several micromolar) triggers $Ca^{2+}$ channel opening. Once opened, the myoplasmic $Ca^{2+}$ concentration increases, which subsequently leads to channel closure. As the $Ca^{2+}$ is pumped back into the SR, the $Ca^{2+}$ concentration should again pass through this optimal $Ca^{2+}$ concentration, at which point the $Ca^{2+}$ channel should again open. This clearly does not happen within the cell. Whatever triggers the opening of the $Ca^{2+}$ channel must be either removed rapidly from the T-tubule–SR junction, or the release protein must somehow have a memory and go into a temporarily closed or partially closed state (Schneider *et al.*, 1985; Maylie *et al.*, 1987) shortly after $Ca^{2+}$ release is triggered. SH–disulfide interchange results in a closed state that cannot immediately interact with the initial trigger.

3. The third step involves overall reduction of the system to its initial reduced configuration. This step may be enzyme controlled. It might be expected to be stimulated by ATP and micromolar $Ca^{2+}$, and inhibited by millimolar $Mg^{2+}$.

The model presented is speculative. It is, however, entirely compatible with our observations on SH-induced $Ca^{2+}$ release, and those of others on $Ca^{2+}$-induced $Ca^{2+}$ release. Release of $Ca^{2+}$ caused by increasing myoplasmic free $Ca^{2+}$ ($10^{-8}$–$10^{-6}$) can be explained by $Ca^{2+}$ ions interacting with $SH_1$ and $SH_2$ to form a $Ca^{2+}$ dithiolate complex or by $Ca^{2+}$, either directly or indirectly, controlling the rate of the oxidation reaction. Still higher $Ca^{2+}$ concentrations may interact with $SH_3$, and affect the closing of the release channel.

At present, not enough is known about SH control of the SR $Ca^{2+}$ permeability and its effect on the gating characteristics of the isolated

reconstituted $Ca^{2+}$ release protein. We have shown that addition of micromolar concentrations of various SH-oxidizing agents stimulates rapid $Ca^{2+}$ release via a pathway that appears to be identical to the $Ca^{2+}$-induced release pathway. We have also shown that transport is inhibited by reduction of the disulfide formed. The SH interaction is reversible. To obtain physiologically relevant $Ca^{2+}$ release rates from SH oxidation and reduction, it is likely that endogenous enzymes control the redox state of these SH groups. The model presented is not meant to be the last word on SH interactions and the $Ca^{2+}$ release protein from the SR. It is consistent with our observations, and is meant to be a working model that should aid in the design of future experiments and enhance our understanding of this critical step in excitation–contraction coupling.

## Acknowledgments

We would like to express our gratitude to the contributions made by Ms. Janice Stuart, Mr. Edmond Buck, and Drs. Jonathan Trimm and Joseph Cronin.

This work was supported by American Heart Association grants 87915 to J.J.A. and 871065 to G.S., and by grants from the Oregon Affiliate of the American Heart Association to J.J.A. and from the Western Pennsylvania Heart Association to G.S. J.J.A. is an Established Investigator of the American Heart Association (84130). G.S. is a recipient of Research Career Development Award K0400909 from the National Institutes of Health.

This paper is Portland State University Environmental Sciences and Resources Program Publication 226.

## References

Abramson, J. J., Buck, E., Salama, G., Casida, J. E., and Pessah, I. N. (1988*a*). *J. Biol. Chem.* **263**, 18750–18758.

Abramson, J. J., and Salama, G. (1988). *Mol. Cell. Biochem.*, **82**, 81–84.

Abramson, J. J., Trimm, J. L., Weden, L., and Salama, G. (1983). *Proc. Natl. Acad. Sci. USA* **80**, 1526–1530.

Abramson, J. J., Cronin, J., and Salama, G. (1988*b*). *Arch. Biochem. Biophys.* **263**, 245–255.

Adunyah, S. E., and Dean, W. L. (1986). *J. Biol. Chem.* **261**, 13,071–13,075.

Aoki, T., Oba, T., and Hotta, K. (1985). *Can. J. Physiol. Pharmacol.* **63**, 1070–1074.

Beatrice, M. C., Stiers, D. L., and Pfeiffer, D. R. (1984). *J. Biol. Chem.* **259**, 1279–1287.

Bindoli, A., and Fleischer, S. (1983). *Arch. Biochem. Biophys.* **221**, 458–466.

Brocklehurst, K. (1979). *J. Biochem.* **10**, 259–274.

Cavallini, D., de Marco, C., Dupre, S., and Rotillio, G. (1969). *Arch. Biochem. Biophys.* **130**, 354–361.

Crane, F. L. (1968). In *Biological Oxidations* (Singer, T. P., ed.), Wiley, New York, pp. 533–580.

Doroshow, J. H. (1983). *Cancer Res.* **43**, 460–472.

Endo, M., Tanaka, M., and Ogawa, Y. (1970). *Nature* **228**, 34–36.
Erlichman, J., Schubart, U., and Fleischer, N. (1979). *Endocrinology* **105**, 1185–1190.
Fabiato, A., and Fabiato, F. (1977). *Circ. Res.* **40**, 119–129.
Ford, L. E., and Podolsky, R. J. (1972). *J. Physiol. (Lond.)* **223**, 21–33.
Hymel, L., Inui, M., Fleischer, S., and Schindler, H. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 441–445.
Imagawa, T., Smith, J. S., Coronado, R., and Campbell, K. P. (1987). *J. Biol. Chem.* **262**, 16,636–16,643.
Inui, M., Saito, A., and Fleischer, S. (1987). *J. Biol. Chem.* **262**, 1740–1747.
Kosower, E. M., and Werman, R. (1971). *Nature [New Biol.]* **233**, 121–123.
Lai, F. A., Erickson, H. P., Rousseau, E., Liu, Q.-Y., and Meissner, G. (1988). *Nature* **331**, 315–319.
Martonosi, A., and Feretos, R. (1964). *J. Biol. Chem.* **239**, 648–658.
Maylie, J., Irving, M., Sizto, N.-L., and Chandler, W. K. (1987). *J. Gen. Physiol.* **89**, 83–143.
Meissner, G. (1984). *J. Biol. Chem.* **258**, 2365–2374.
Meissner, G., Darling, E., and Eveleth, J. (1966). *Biochemistry* **25**, 236–244.
Moutin, M. J., Abramson, J., Salama, G., and Dupont, Y. (1988). *Biophys. J.* **53**, 136a.
Pessah, I. N., Francini, A. O., Scales, D. J., Waterhouse, A. L., and Casida, J. E. (1986). *J. Biol. Chem.* **261**, 8643–8648.
Pessah, I. N., Stambuk, R. A., and Casida, J. E. (1987). *Mol. Pharmacol.* **31**, 232–238.
Pike, G., Abramson, J., and Salama, G. (1987). *Biophys. J.* **51**, 106a.
Salama, G., and Abramson, J. (1984). *J. Biol. Chem.* **259**, 13,363–13,369.
Salama, G., Pike, G., Fuchs, F., and Abramson, J. (1986). *Biophys. J.* **49**, 560a.
Salama, G., Zaidi, N. F., Abramson, J. J., and Lagenaur, C. (1988). *Biophys. J.* **53**, 420a.
Schneider, M. F., and Chandler, W. K. (1973). *Nature* **242**, 244–246.
Schneider, M. F., Rios, E., and Melzer, W. (1985). *Cell Calcium* **6**, 109–118.
Shamoo, A. E., and MacLennan, D. H. (1975). *J. Membr. Biol.* **25**, 65–74.
Stuart, J., and Abramson, J. J. (1988). *Arch. Biochem. Biophys.*, **264**, 125–134.
Thor, H., Hartzell, P., Swensson, S., Orrenius, S., Mirabelli, F., Marinoni, V., and Bellamo, G. (1985). *Biochem. Pharmacol.* **34**, 3717–3723.
Trimm, J. L. Salama, G., and Abramson, J. J. (1986). *J. Biol. Chem.* **261**, 16,092–16,098.
Williams, C. H. (1976). In *The Enzymes* (Boyer, P. D., ed.), Vol. 13, Part C, 3rd ed., Academic Press, Orlando, Florida, p. 94.
Zaidi, N. F., Abramson, J. J., Lagenaur, C., and Salama, G. (1988). *Biophys. J.* **53**, 456a.
Zorzato, F., Salviati, G., Facchinetti, T., and Volpe, P. (1985). *J. Biol. Chem.* **260**, 7349–7355.
Zorzato, F., Margreth, A., and Volpe, P. (1986). *J. Biol. Chem.* **261**, 13,252–13,257.