Linking Calsequestrin to Lumenal Control of SR Ca\textsuperscript{2+} Release

Thomas R. Shannon

_Circ Res._ 2007;101:539-541
doi: 10.1161/CIRCRESAHA.107.160952

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 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/101/6/539

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/
Influx of Na, a small Ca\(^{2+}\) influx across the sarcolemma causes a large release of Ca\(^{2+}\) from the sarcoplasmic reticulum (SR). Release of this Ca\(^{2+}\) takes place via the ryanodine receptor (RyR), a Ca\(^{2+}\) channel within the SR membrane which is gated by cytoplasmic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{c}\)). This gating takes place to a small but significant extent even at the relatively low [Ca\(^{2+}\)], found during diastole within the myocyte (SR Ca\(^{2+}\) leak).

Among the striking features of this SR Ca\(^{2+}\) release is its steep nonlinear dependence on the total concentration of Ca\(^{2+}\) found in the lumen of the SR ([Ca\(^{2+}\)]\(_{SR}\)). The degree of release at diastolic [Ca\(^{2+}\)], is very low when the [Ca\(^{2+}\)]\(_{SR}\) is approximately 50% of the level usually found in an isolated cardiac myocyte. However, it increases dramatically as [Ca\(^{2+}\)]\(_{SR}\) increases toward its normal level.

**Functional Consequences of Luminal Ca\(^{2+}\) Regulation of SR Release**

Of special interest is the effect of SR [Ca\(^{2+}\)] on the SR Ca\(^{2+}\) leak through the RyR. In the cardiac ventricular myocyte, this leak takes place just under the sarcolemmal membrane, the location of many proteins whose effects are regulated by Ca\(^{2+}\). The magnitude of SR Ca\(^{2+}\) leak may alter the activity of these proteins. Among the candidate proteins which may be affected by the subsarcolemmal Ca\(^{2+}\) is the sodium-calcium exchanger (NCX). This may be particularly relevant in cells from hearts undergoing chronic failure because the myocytes from these heart have a higher leak rate than normal. These cells already exhibit increased NCX expression and decreased inward rectifier current. The 3 components added together may result in electrical destabilization of the sarcolemmal membrane such that depolarization and delayed afterdepolarizations may result. This instability may increase the tendency of failure hearts to exhibit arrhythmias.

Defective SR luminal Ca\(^{2+}\) regulation of release also increases SR Ca\(^{2+}\) leak and may increase the tendency to generate arrhythmias in this manner. For instance, humans who are homozygous for nonsense calsequestrin (CSQ) mutations exhibit catecholaminergic polymorphic ventricular tachycardia (CPVT). The reason why catecholamines increase the tendency to demonstrate this arrhythmia is still a matter of debate.

**The Mechanism of SR Luminal Ca\(^{2+}\) Release Regulation**

To better understand these effects the mechanisms by which luminal Ca\(^{2+}\) controls SR Ca\(^{2+}\) release must be considered. Generally speaking there are 2 possibilities which are not necessarily mutually exclusive: that the free SR [Ca\(^{2+}\)] ([Ca\(^{2+}\)]\(_{SR}\)), itself, has a direct effect on the process of SR Ca\(^{2+}\) release or that this effect is mediated via another luminal substance, most likely CSQ, the major SR luminal Ca\(^{2+}\) binding protein.

**Free SR [Ca\(^{2+}\)]**

The simplest mechanism by which luminal control of SR Ca\(^{2+}\) release could take place is through a direct effect of [Ca\(^{2+}\)]\(_{SR}\) on the flux. The Ca\(^{2+}\) flux through a single RyR rises and falls linearly with the free [Ca\(^{2+}\)] gradient across the SR membrane. However, it is possible that the RyR has a luminal binding site for Ca\(^{2+}\). Ca\(^{2+}\) binding to this site might alter the release process itself, by increasing the probability of opening at any given [Ca\(^{2+}\)]. (Figure A).

**Regulation by Calsequestrin**

If the effect of luminal Ca\(^{2+}\) is not mediated directly by the [Ca\(^{2+}\)]\(_{SR}\), then the effect might be mediated by a Ca\(^{2+}\) sensing protein located within the SR lumen. Of the proteins present, CSQ would appear to be a prime candidate. It is known to interact with the RyR in a Ca\(^{2+}\)-dependent manner. This interaction may be direct but also may be indirect through the mediation of triadin or junction (Figure, bottom). The last of these deserves closer attention.

The most well supported model of this type is that proposed by the Gyorke-Jones group. According to this theory, CSQ is normally bound to the RyR through junctin or triadin at low [Ca\(^{2+}\)]\(_{SR}\). The P\(_o\) of the RyR channel is, therefore, correspondingly low in planar lipid bilayers at low trans (ie, SR luminal). As [Ca\(^{2+}\)]\(_{SR}\) rises, CSQ dissociates from the receptor and increases the P\(_o\) of the channel (and thus SR Ca\(^{2+}\) leak) via release of inhibition.

**Addressing the Molecular Mechanism Experimentally**

The article by Chopra et al in this issue of Circulation Research differentiates between these possibilities. This group has approached the dilemma previously by evaluating...
The beauty of the study in the current issue is that it uses heart which accompany the cellular changes. Though the typical tendency is to consider heterozygote transgenic animals to be an unwanted side effect of the the effects of completely knocking out the gene for cardiac CSQ in mice. Somewhat surprisingly, isolated cardiac myocytes from these CSQ-null mice (CSQ<sup>−/−</sup>) displayed normal SR Ca<sup>2+</sup> release under basal conditions even though upregulation of other Ca<sup>2+</sup> binding proteins was not apparent. The mice adapted to the loss through a striking increase in SR volume. Thus the [Ca<sup>2+</sup>]<sub>SR</sub> was unchanged despite the decreased buffering capacity of the organelle. Significantly, exposure to catecholamines in CSQ<sup>−/−</sup> myocytes caused increased diastolic SR Ca<sup>2+</sup> leak, leading to premature spontaneous SR Ca<sup>2+</sup> releases and triggered beats. The results implied that the lack of functional CSQ led to dysregulation of the RyR by luminal SR Ca<sup>2+</sup> and that the mice were, therefore, more susceptible to CPVT.

There were, however, characteristics of the CSQ<sup>−/−</sup> phenotype which limited the conclusions which could be drawn from the study. Chief among these was a change in the expression of other SR proteins such as triadin and junctin in the phenotype, making it very difficult to attribute the luminal dysregulator of the RyR to the absence of CSQ. In addition, although the phenotype displayed the increase in SR Ca<sup>2+</sup> leak, and therefore RyR activity, the effects could not differentiate between an effect of the CSQ on the RyR and the effect of the decrease in buffering capacity within the SR lumen.

One thing was, however, very clear. The total absence of CSQ did not eliminate the dependence of the SR Ca<sup>2+</sup> leak on luminal SR [Ca<sup>2+</sup>]<sub>SR</sub>. The leak, though higher at any given [Ca<sup>2+</sup>]<sub>SR</sub>, continued to increase with luminal [Ca<sup>2+</sup>]<sub>i</sub> in a steeply nonlinear fashion. It was apparent that whatever the effect of CSQ on release, the whole story would likely involve other players, perhaps including direct actions of the [Ca<sup>2+</sup>]<sub>SR</sub> on the process as described above.

The beauty of the study in the current issue is that it uses the unique characteristics of the mice which are heterozygous for the CSQ2 deletion (CSQ<sup>−/+</sup>) to take the results from the previous study further. These mice exhibited a much milder form of the phenotype with only a 25% decrease in CSQ2 expression. This change was not severe enough to induce a change in SR volume and, though the functional [Ca<sup>2+</sup>]<sub>SR</sub> measured by the height of caffeine transients was not different from wild-type in unchallenged myocytes, it was lower in isoproterenol-challenged myocytes.

Perhaps more importantly, there was no change in the levels of either junctin or triadin compared with wild-type in the CSQ<sup>−/−</sup> mice. Despite this, an increase in SR Ca<sup>2+</sup> leak was still detected. Indeed, despite the relatively mild phenotype in the presence of isoproterenol the mice continued to exhibit higher rates of ventricular ectopy and significantly higher rates of spontaneous SR Ca<sup>2+</sup> release and triggered beats in isolated ventricular myocytes. These results strengthen the CSQ<sup>−/−</sup> results above by eliminating the possibility that the decrease in junctin/triadin played a role in the susceptibility to CPVT.

The major remaining mechanistic problem was that the authors could not distinguish between the possibility that the CSQ was interacting with the RyR to change its behavior from the possibility that the altered buffering capacity of the SR was responsible. In other words, though the SR Ca<sup>2+</sup> leak was higher at any given [Ca<sup>2+</sup>]<sub>SR</sub>, they could not tell whether a higher [Ca<sup>2+</sup>]<sub>SR</sub>, rather than the reduced CSQ was responsible. To differentiate between these possibilities, the authors measured the SR Ca<sup>2+</sup> leak and the [Ca<sup>2+</sup>]<sub>SR</sub> simultaneously (Figure). The SR Ca<sup>2+</sup> leak remained higher in the CSQ<sup>−/−</sup> at the same [Ca<sup>2+</sup>]<sub>SR</sub> thus supporting the hypothesis that the CSQ was interacting with and regulating the RyR even at the same [Ca<sup>2+</sup>]<sub>SR</sub>.

These data go a long way toward validating the theory that CSQ regulates SR Ca<sup>2+</sup> release through the RyR. They complement previous experimental data using adult ventricular myocytes which were transfected with antisense cDNA to reduce CSQ levels. The technique used here has significant advantages, however, in that the authors have avoided the potential artifacts associated with culturing cardiac myocytes and have measured electrical anomalies in the whole heart which accompany the cellular changes.

Though the typical tendency is to consider heterozygote transgenic animals to be an unwanted side effect of the...
production of more “pure” homozygote animals, the manuscript emphasizes the importance of considering all of the tools available in an effort to produce a picture which may at first seem less cut and dried but which may be in the end more complete.

**Remaining Questions**

There are still numerous questions regarding the luminal regulation of the process of release which remain to be answered. Mechanistically, the fact that the load-dependence of release remains in the absence of CSQ indicates that the picture is likely to be complicated. What this residual effect is attributable to can only be guessed at but the role which the \([\text{Ca}^{2+}]_{\text{sa}}\) itself plays, and how, remain to be considered.

A related issue to be resolved is the luminal \(\text{Ca}^{2+}\)-dependence of the CSQ mediated regulation. Clearly the effect of CSQ on the RyR is \(\text{Ca}^{2+}\)-dependent both in \(\text{Ca}^{2+}\) overlay experiments and in planar lipid bilayers. Its dependence in intact myocytes under physiological conditions and at physiological luminal SR \([\text{Ca}^{2+}]\) remains unclear.

Finally, though CSQ affects the process of diastolic release, the effect of CSQ on the mechanism of active release remains unclear. This determination is complicated by the numerous processes involved in excitation contraction coupling and \(\text{Ca}^{2+}\) homeostasis in the cardiac myocyte. The most critical mechanistic question regards the role of the luminal \(\text{Ca}^{2+}\) in the termination of SR \(\text{Ca}^{2+}\) release.\(^9\) For instance, as the SR \([\text{Ca}^{2+}]\) falls during contraction, could the CSQ act dynamically within the relevant timeframe to reduce the probability of opening of the RyR such that it closes rather than continuing as a regenerative process? If so, does it accomplish this by switching off \(\text{Ca}^{2+}\)-dependent cytosolic activation or by facilitating a cytosolic inactivation mechanism?

Although these questions remain unanswered, it is clear that with well thought out and well executed studies such as the present, we can expect to address them in a fashion which will eventually give reasonable and relevant results. Such data will lead to better ways to target the process of SR \(\text{Ca}^{2+}\) release to more effectively treat cardiac arrhythmias.

**Sources of Funding**

T.S. is supported by NIH R01 HL071893-04.

**Disclosures**

None.

**References**


**Key Words:** adrenergic stimulation  ■ arrhythmia  ■ \(\text{Ca}^{2+}\) handling  ■ ryanodine receptor  ■ sarcoplasmic reticulum