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EDITORIAL

Direct Modulation of RyR2 Leading to a TRICky Ca^{2+} Balance

The Effects of TRIC-A on Cardiac Muscle

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A central point in heart research is to understand the mechanisms behind Ca^{2+} handling and to find alternatives to correct defective Ca^{2+} cycling associated with heart failure and arrhythmias. In cardiac myocytes, entry of Ca^{2+} occurs through L-type Ca^{2+} channels and mediates the opening of RyR2 (ryanodine receptor 2) channels allowing systolic movement of Ca^{2+} from the sarcoplasmic reticulum (SR) to the cytosol. In addition, SR Ca^{2+} release occurs randomly due to RyR2 opening in response to a combination of factors that favor (luminal and cytosolic Ca^{2+}, ATP, posttranslational modifications; gain-of-function mutations or drugs) or preclude it (Mg^{2+}, FKBPs, loss-of-function mutations or drugs). Due to its dependence on luminal SR Ca^{2+}, the concept of SOICR (store overload-induced SR Ca^{2+} release) has emerged, and the capability of these events to generate delayed afterdepolarizations and arrhythmias is widely accepted.[1] Thus, understanding the mechanisms that regulate SR Ca^{2+} release and its capacity to promote SOICR is of critical importance.

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In every heartbeat, the Ca^{2+} current passing through the SR membrane is considered to need a counterbalance current to maintain SR membrane voltage. Yazawa et al[2] originally unveiled the molecular identity of the K^+ permeable SR counterion channels as subtypes of trimeric intracellular cation channels (TRICs). These monovalent cation-selective channels have 3 transmembrane segments. The amino terminus domain is located in the SR/ER (endoplasmic reticulum) lumen, whereas the carboxy terminus is exposed to the cytoplasm. TRIC-A is preferentially expressed in excitable cells, whereas TRIC-B is present in most mammalian tissues.[3]

In this issue of *Circulation Research*, Zhou et al[4] demonstrate a direct effect of TRIC-A on RyR2 gating, which impacts on Ca^{2+} handling, modulates SOICR, and protects against isoproterenol-mediated cardiac damage. Using cardiac myocytes from mice carrying a deletion for TRIC-A (TRIC-$A^{-/-}$), they show that compared with WT (wild type) myocytes, TRIC-$A^{-/-}$ cells have a lower basal Ca^{2+} spark frequency and a higher SR Ca^{2+} content. Furthermore, they show that TRIC-$A^{-/-}$ myocytes have slower rising and prolonged intracellular Ca^{2+} transients and reduced SOICR compared with WT suggesting that TRIC-A ablation leads to a reduction in RyR2 channel activity. Confirming the role of TRIC-A as a modulator of RyR2 function, coexpression of RyR2 and TRIC-A in HEK293 cells leads to a decrease in spontaneous Ca^{2+} oscillations and a decrease in SOICR as a result of reduced ER Ca^{2+} content. These effects are not observed when RyR2 is coexpressed with TRIC-B. From these results, it can be concluded that TRIC-A constitutes a physiological component of the SR Ca^{2+} release machinery and that TRIC-A deficiency could render RyR2 channels less sensitive to physiological activation of Ca^{2+} signaling in cardiac muscle. However, as we will discuss further below, this conclusion is not straight forward as both TRIC-$A^{-/-}$ and RyR2 coexpressed with TRIC-A are associated with a reduction in SOICR.

Having convincingly established that TRIC-A and not TRIC-B modulates RyR2 channel function, the question that arises is "how does TRIC-A modulate RyR2?" Topology

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analysis has shown that both TRIC-A and TRIC-B contain a carboxyl-terminal tail (CTT) domain that resides in the cytosol. CTT from TRIC-A contains a histidine-rich motif and a polylysine domain flanked by a hydrophobic domain. This structure is similar to the intracellular loop of the L-type Ca^{2+} channel, which has been shown to regulate SR Ca^{2+} release in muscle cells and thus emerges as a putative candidate motif for the regulation of RyR2 in cardiac cells. Elegant experiments performed by Zhuo et al using chimeric constructs containing either the CCT from TRIC-A or B coexpressed with RyR2 in HEK293 cells showed that replacement of the CTT domain in TRIC-A with CTT-B could prevent the reduction of SOICR observed when native TRIC-A is overexpressed, highlighting the important function of CTT-A on RyR2-mediated SOICR. More direct evidence of the impact of CCT-A on RyR2 function was provided by measurements of single-channel activity of RyR2 reconstituted in lipid bilayers. In contrast to CCT-B, the addition of CCT-A significantly enhanced RyR2 opening. Taken together, the authors provide solid support for a novel function of TRIC-A as a regulatory protein that by interacting with RyR2 through its CCT domain modulates Ca^{2+} handling and SOICR (Figure).

Regarding the TRICky Ca^{2+} balance referred to in the tittle, the following deserves to be analyzed: the lack of TRIC-A could be considered a loss-of-function modulation of the RyR2 complex given that TRIC-$A^{-/-}$ myocytes show a decrease in SOICR resulting in a higher SR Ca^{2+} load. However, experiments in HEK293 cells and in cardiac myocytes microinjected with TRIC-A (gain of function) also show a reduction in SOICR as a consequence of a reduction in SR Ca^{2+} load. Thus, why does a gain-of-function modulation of RyR2 results in reduced SOICR?

In HEK293 cells, this can be attributed to a disorganized Ca^{2+} leak that precludes the development RyR2 recruitment and macroscopic SOICR. In fact, nonspark-mediated Ca^{2+} leak has been recognized as a variety of Ca^{2+} spillover that can deplete SR Ca^{2+} content without eliciting Ca^{2+} release events such as sparks or waves detectable by regular confocal microscopy.[5] In cardiac myocytes, a transient rise in Ca^{2+} spark frequency promoted by TRIC-A is in fact the cause of partial SR depletion resulting in reduced SOICR. The question is "Can TRIC-A reduce SOICR and arrhythmias under any physiological condition?" We speculate not or at least the data do not completely support such paradigm. The final outcome will strongly depend on SR Ca^{2+} load, which is not only determined by RyR2-dependent Ca^{2+} leak but could rise during adrenergic stimulation, rapid pacing, or interventions that increase cytosolic Ca^{2+}. As the authors discussed, previous work from Chen et al[6] showed that RyR2 gain-of-function mutants could promote SOICR. We could consider that under conditions that promote SR Ca^{2+} loading, TRIC-A bound RyR2 should behave similar to other forms of RyR2 activation such as mutations or phosphorylation, which elicit SOICR when SR load is high. Indeed, in failing cardiomyocytes in which RyR2 dysfunction mediates SR depletion and contractile impairment, the risk of arrhythmia persists.[7]

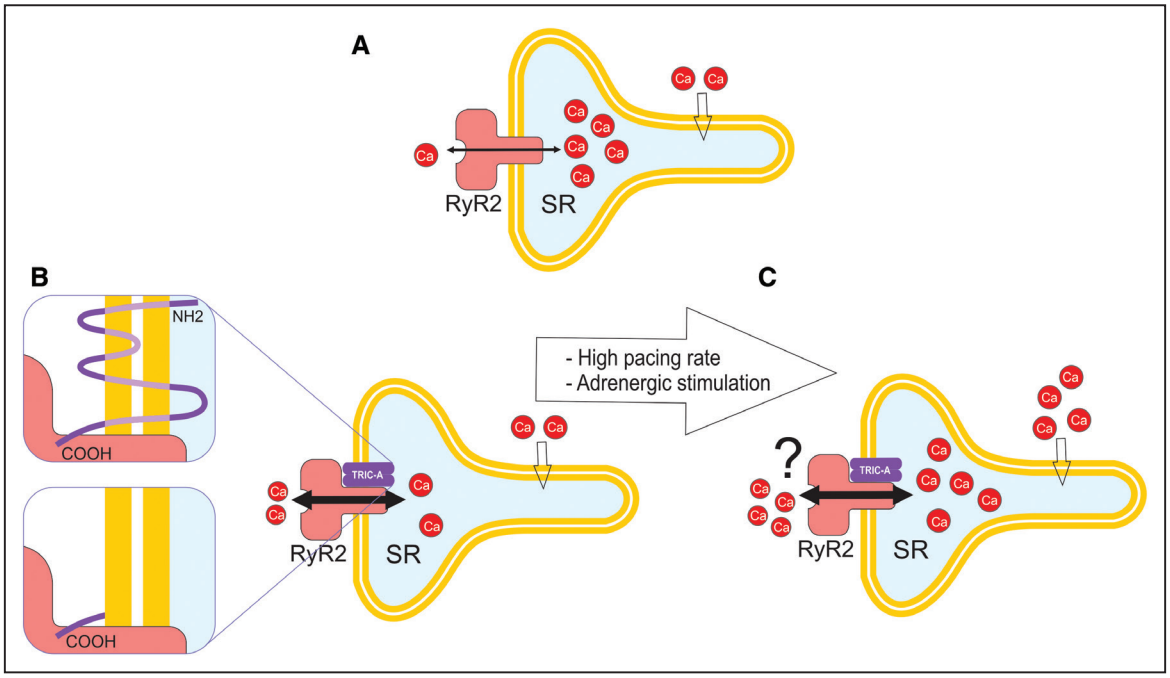

Figure. Impact of trimeric intracellular cation channel (TRIC)-A (or CTTA)/RyR2 (ryanodine receptor 2) interaction on sarcoplasmic reticulum (SR) calcium handling.

Schematic representation comparing SR Ca^{2+} handling in the presence and absence of TRIC-A. **A**, In the absence of TRIC-A, RyR2 opening is depressed leading increased SR Ca^{2+} content. In **B** instead, the interaction of both full TRIC-A or its carboxi-terminus tail (above and below in the inset, respectively) boosts RyR2 gating, promoting SR Ca^{2+} depletion, which reduces store overload–induced SR Ca^{2+} release (SOICR). **C**, Putative impact of RyR2/TRIC-A interaction on SOICR during physiological conditions that load the SR with Ca^{2+}.

Interestingly, in this issue, Zhou et al showed that TRIC-A$^{-/-}$ mice are more susceptible to isoproterenol-induced arrhythmia and fibrosis, but no mechanistic explanation was provided. There is lack of data showing Ca^{2+} spark and wave frequency under isoproterenol challenge. Thus, whether ECG-detected arrhythmia in TRIC-A$^{-/-}$ mice is due to the occurrence of SOICR it is not straight forward. In fact, one would expect isoproterenol-induced SOICR to be enhanced in TRIC-A–expressing cells rather than in TRIC-A$^{-/-}$ cells. Nevertheless, the findings of Zhou et al[8] are not completely unexpected considering that previous reports have shown that RyR2 suppression of function leads to SR Ca^{2+} overload and ultimately arrhythmias. A parallelism between TRIC-A$^{-/-}$ and A4860G mutants (RyR2 loss-of-function CPVT model) can be made, but, if this were the case, the development of postsystolic Ca^{2+} release and early afterdepolarizations instead of the classical delayed afterdepolarizations associated with SOICR would be expected.

A possible explanation for isoproterenol-induced cell toxicity and fibrosis could be that isoproterenol, by favoring a further increase of SR Ca^{2+} load in TRIC-A$^{-/-}$ cells, could promote ER stress leading to cytotoxicity and fibrosis. This hypothesis is consistent with a recent report showing that isoproterenol promotes ER stress and myocyte death via a Ca^{2+}-dependent mechanism.[9]

In our opinion, this article opens a whole set of interesting questions:

1. Does TRIC-A affect RyR2 spatial organization within the SR? In the last few years, the importance of RyR2 clustering (spatial organization within the SR membrane) on SR Ca^{2+} handling has been recognized.[10] Thus, whether TRIC-A affects RyR2 clustering would be an interesting possibility to explore.
2. What is the proportion of TRIC-A bound to RyR2? Other proteins that regulate RyR2 activity are not bound at a hundred percent with RyR2, and this proportion would be key to understand the physiological impact of TRIC-A on cardiac function.
3. Are there changes in TRIC-A expression and in TRIC-A/RyR2 interaction during pathological cardiac remodeling? If these changes occur, they could have important consequences on SOICR and arrhythmogenesis.
4. How does TRIC-A affect Ca^{2+} handling at high pacing rates? It is well known that high pacing rates are associated with alternans induction.[11] Zhong et al[12] have demonstrated that RyR2 loss of function can facilitate the development of Ca^{2+} alternans, and this can be prevented by caffeine due to its capacity to increase RyR2 opening. In this context, it seems worth knowing whether or not TRIC-A prevents Ca^{2+} alternans development.

Overall, in the cardiovascular field, there is an unmet need for novel therapeutic approaches that correct SR Ca^{2+} handling in different pathological situations, and RyR2 Ca^{2+} release complex is a potential target for this. The present study sheds light on a novel regulation of RyR2 by TRIC-A and will surely motivate future research leading to a better modulation of SR Ca^{2+} handling and arrhythmia prevention.

ARTICLE INFORMATION

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Disclosures

None.

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