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Impact of RyR2 potentiation on myocardial function

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Lascano E, Negroni J, Vila Petroff M, Mattiazzi A. Impact of RyR2 potentiation on myocardial function. *Am J Physiol Heart Circ Physiol* 312: H1105–H1109, 2017. First published April 7, 2017; doi:10.1152/ajpheart.00855.2016.—This perspective attempts to shed light on an old and not yet solved controversy in cardiac physiology, i.e., the impact of increasing ryanodine receptor (RyR)2 open probability on myocardial function. Based on an already proven myocyte model, it was shown that increasing RyR2 open probability results in a purely short-lived increase in Ca^{2+} transient amplitude, and, therefore, it does not increase cardiac contractility. However, potentiation of RyR2 activity permanently enhances fractional Ca^{2+} release, shifting the intracellular Ca^{2+} transient versus sarcoplasmic reticulum (SR) Ca^{2+} content curve to a new state of higher efficiency. This would allow the heart to maintain a given contractility despite a decrease in SR Ca^{2+} content, to enhance contractility if SR Ca^{2+} content is simultaneously preserved or to successfully counteract the effects of a negative inotropic intervention.

NEW & NOTEWORTHY Increasing ryanodine receptor (RyR)2 open probability does not increase cardiac contractility. However, RyR2 potentiation shifts the intracellular Ca^{2+} transient-sarcoplasmic reticulum (SR) Ca^{2+} content relationship toward an enhanced efficiency state, which may contribute to a positive inotropic effect, preserve contractility despite decreased SR Ca^{2+} content, or successfully counteract the effects of a negative inotropic action.

heart; contractility; ryanodine receptor; open probability

> ". . . it has not been generally appreciated that a single Starling curve cannot always satisfactorily explain the observed phenomena; for any given heart there is a series or family of curves."
>
> Sarnoff and Berglund, 1954 (26)

SARCOLEMMAL DEPOLARIZATION during each cardiac cycle initiates cardiac excitation-contraction coupling (ECC): Ca^{2+} influx through voltage-gated L-type Ca^{2+} channel current (I_{Ca}) activates ryanodine receptor (RyR)2 Ca^{2+}-release channels in the sarcoplasmic reticulum (SR), allowing the release of a much large amount of Ca^{2+} from the SR into the cytosol via the Ca^{2+}-induced Ca^{2+}-release (CICR) mechanism (9). This mechanism gives rise to a transitory increase in intracellular Ca^{2+} (Ca^{2+} transient) that signals contractile myofilaments to generate force and shortening. Relaxation occurs when Ca^{2+} returns to the diastolic Ca^{2+} level, mainly due to the termination of SR Ca^{2+} release in association with the activity of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)2a and, to a lesser extent, the sarcolemma Na^+/Ca^{2+} exchanger (NCX) working in the forward mode (1, 5).

The CICR mechanism is therefore essentially an amplifying process by which a modest Ca^{2+} triggering signal is significantly magnified. This is often referred to as gain (18, 28), i.e., the ratio between SR Ca^{2+} released and I_{Ca}. Moreover, for a given Ca^{2+} triggering signal, the SR releases a fraction of its Ca^{2+} content [fractional SR Ca^{2+} release (FCaR)], which has been shown to be dependent on SR Ca^{2+} load (18). Together with ECC gain, FCaR has been used as a complementary numerical index of ECC efficacy (10). Whereas an increase in either I_{Ca} or SR Ca^{2+} content are well-established positive inotropic mechanisms (16, 17, 27, 35), the increase in the open probability (P_o) of RyR2 cannot be easily related to an improvement in cardiac function. This is in part because assessment of the functional outcome of RyR2 potentiation in intact cells is challenging. For instance, the simultaneous increase in I_{Ca} and SR Ca^{2+} uptake and load produced by β-adrenoceptor (β-AR) stimulation (10, 16) will independently increase SR Ca^{2+} release, hampering the dissection of the contribution of RyR2 phosphorylation per se, if any, to the positive inotropic action of β-AR stimulation. To circumvent this problem, SR Ca^{2+} load and I_{Ca} have to be maintained constant (10, 18). The general outcome of this type of experiments indicates that whereas PKA-dependent phosphorylation of RyR2 has little effect on ECC in a physiological milieu, Ca^{2+}/calmodulin-dependent protein kinase (CaMK)II-dependent phosphorylation of RyR2 increases Ca^{2+}-release channel activity in intact cardiac myocytes during ECC. This phosphorylation ". . . would produce a change in the sensitivity of the RyR2 to activation by Ca^{2+}, such that a greater SR Ca^{2+} efflux occurs for a given I_{Ca}" (18). These conclusions are important, because they suggest a functional role of RyR2 phosphorylation in addition to the recognized detrimental effect of CaMKII-dependent phosphorylation-induced passive SR Ca^{2+} leak and arrhythmia susceptibility (8, 22, 33).

To further assess this issue, we used an already proven human myocyte mathematical model in which the effect of an increase in RyR2 P_o on myocardial function was mimicked by increasing RyR2 conductance (22). The model faithfully reproduces the experimental behavior of myocytes from wild-type (WT) mice and myocytes from S2814D mice, with increased P_o produced by constitutive pseudo-phosphorylation of RyR2 Ser^{2814} (33). Figure 1 shows that increasing RyR2 conductance by 50% did not have a sustained effect on the

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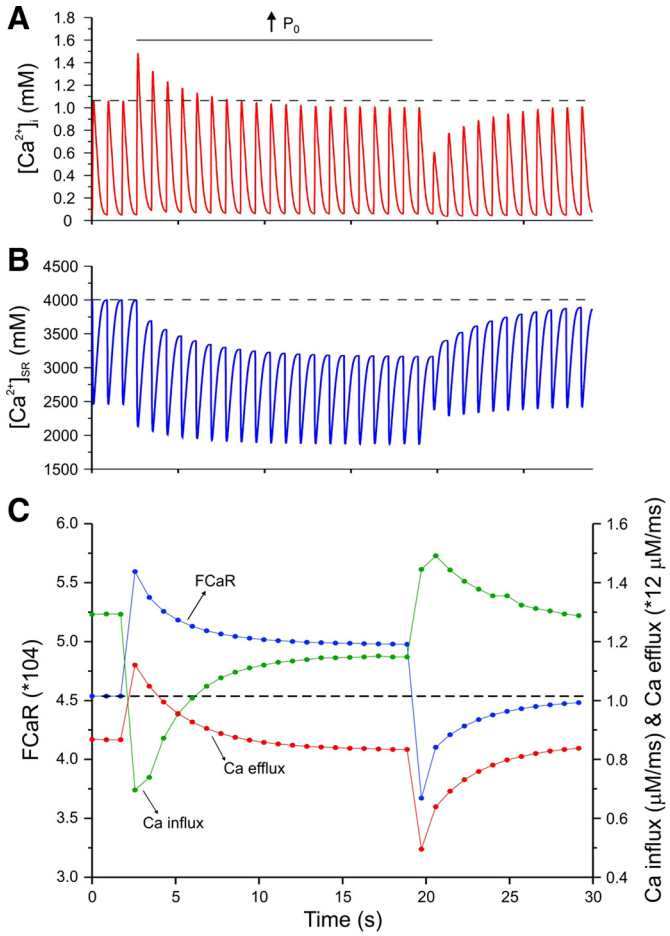

Fig. 1. Time course of sarcolemmal and sarcoplasmic reticulum (SR) Ca^{2+} fluxes produced by increased ryanodine receptor (RyR)2 open probability (P_o). *A*: intracellular Ca^{2+} transient. *B*: SR Ca^{2+} content. *C*: fractional SR Ca^{2+} release (FCaR), Ca^{2+} efflux, and Ca^{2+} influx. The bar indicates the interval in which P_o was increased ($\uparrow P_o$). $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; $[Ca^{2+}]_{SR}$, SR Ca^{2+} concentration.

systolic Ca^{2+} transient. Effectively, and similarly to previous findings obtained with the application of low caffeine concentrations to modestly increase RyR2 P_o (12, 32), it resulted in a purely short-lived increase in Ca^{2+} transient amplitude, in such a way that, when the steady state is reached, the amplitude of the Ca^{2+} transient is virtually identical to that observed under control conditions, even though the increase in the RyR2 P_o is still present. As noted by different reports by Greensmith et al. (12) and Trafford et al. (32), our model indicates that the temporary increase in Ca^{2+} transient when the enhanced RyR2 conductance is applied results in decreased Ca^{2+} entry into the cell and increased Ca^{2+} efflux, producing the subsequent drop in both, SR Ca^{2+} content and the systolic Ca^{2+} transient. Notably, and as shown in Fig. 1*C*, RyR2 potentiation enhanced FCaR in association with the brief increase of the Ca^{2+} transient. After increasing, FCaR decreased by ~50% and remained at this intermediate value, higher than control, until the effect of high P_o was removed. This occurred despite the fact that SR Ca^{2+} load decreased below the levels observed before RyR2 potentiation. Indeed, experiments in myocytes from the above-mentioned Ser2814D mice exhibited enhanced FCaR compared with WT mice for a similar SR Ca^{2+} load (22, 33). These findings indicate that the increase in P_o certainly produces an ephemeral effect on Ca^{2+} transients and it therefore fails to induce a sustained increase in contractility. However, it evokes a long-lasting effect on the systolic SR Ca^{2+}-release mechanism, such that, for a given SR Ca^{2+} content, the SR Ca^{2+} release is increased. The heart becomes more efficient. Is this effect meaningful in the scenario of cardiac function and inotropism?

Let's make a digression from the ECC and consider for a moment the typical family of Sarnoff's ventricular function curves schematically shown in Fig. 2*A*. The "normal" curve depicts how cardiac systolic work increases with left ventricular end-diastolic pressure, taken as an index of resting fiber length (Frank-Starling mechanism). The other two curves (dashed lines) represent a different condition of the heart, in this case a condition of higher and lower inotropic state. An increase in inotropism at a given resting (diastolic) length occurs when going from *point A* to *point B* (vertical arrow) in Fig. 2*A*. The increase in cardiac output would decrease diastolic volume (length), and the ventricular work would shift according to Starling's law to *point C* or *point D* (horizontal arrow) in Fig. 2*A*. In the latter case, the inotropic state would increase without detectable increases in ventricular work (26).

Figure 2*B* shows the nonlinear relationship between the magnitude of the systolic Ca^{2+} transient and SR Ca^{2+} content under physiological "normal" conditions according to the model. This relationship (blue line in Fig. 2*B*), in which the magnitude of the Ca^{2+} transient increases proportionately more than the SR Ca^{2+} content, is comparable with previously reported experimental data (31). The red line in Fig. 2*B* shows a similar relationship in a myocyte with a 50% increase in RyR2 conductance. The effect of increasing P_o produced an upward and leftward shift of the curves. On these curves, we can represent the sequential effect of increasing RyR2 P_o. The vertical arrow, from *point A* to *point B* (in Fig. 2*B*), indicates the increase in Ca^{2+} transient produced by an increase in P_o. The Ca^{2+} transient would then decrease, following the red curve (dashed arrow in Fig. 2*B*), according to the decrease in SR Ca^{2+} content reaching the same control Ca^{2+} transient level (*point C*). Following a criterion similar to that used with the ventricular function curves, one may conclude that both curves represent different functional states of the heart, such that for a given SR Ca^{2+} content, the release of Ca^{2+} is greater in the curve that represents the increased RyR2 P_o.

We believe that the concept may be relevant and not simply semantic, since it may help to clarify and dissect the contribution to cardiac function inherent to an increase in the activity of RyR2 per se in the experimental setting. For instance, we can consider, first, the aforementioned condition of an enhanced CaMKII-dependent phosphorylation of RyR2 (S2814D). In this case, previous reports (22, 33) found that FCaR was higher versus WT myocytes, whereas the amplitude of the Ca^{2+} transient was similar to WT levels despite the decrease in SR Ca^{2+} content. These results strongly suggest that S2814D myocytes can reach WT Ca^{2+} transient amplitude due to the increase in RyR2 P_o produced by CaMKII phosphorylation. Otherwise, their contractility would be lower. Second, there is the situation of a negative inotropic effect associated with an increase in RyR2 P_o. For instance, it has been shown that the negative inotropic effect induced by hypotonic stress (HS) is exacerbated in the presence of inhibition of the cGMP/PKG

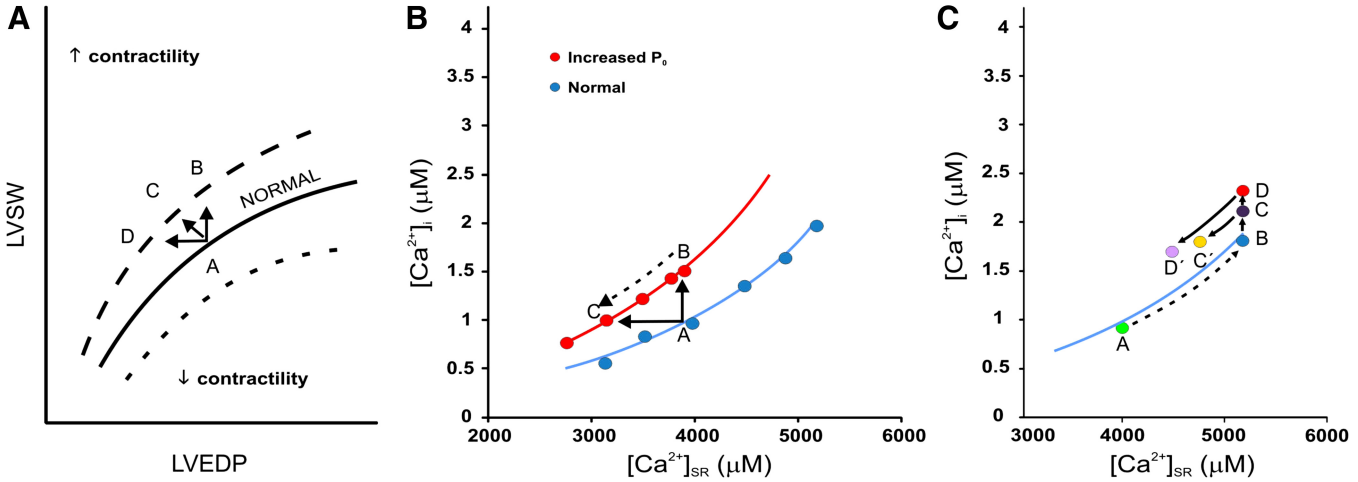

Fig. 2. *A*: typical Sarnoff ventricular function curves. Normal represents the control curve. LVSW, left ventricular systolic work; LVEDP, left ventricular end-diastolic pressure. *B*: steady-state peak $[Ca^{2+}]_i$ as a function of $[Ca^{2+}]_{SR}$. Arrows represent the sequential changes in SR Ca^{2+} load and Ca^{2+} transients after increasing RyR2 conductance by 50% under basal conditions, as described in Fig. 1. *C*: sequential changes in Ca^{2+} transients during β-adrenoceptor stimulation. Increasing L-type Ca^{2+} channel current and sarco(endo)plasmic reticulum Ca^{2+}-ATPase 2a activity increased SR Ca^{2+} load and the Ca^{2+} transient from *point A* to *point B*. Arrows from *points B* to *C* and *C′* and *D* and *D′* represent the successive changes in Ca^{2+} transients after increasing P_o by 15% and 25%, respectively.

pathway, which, during HS, is responsible for PKG-dependent phosphorylation of RyR2, sensitizing RyR2 to Ca^{2+} (11). In this case, the increase in SR Ca^{2+} release produced by this phosphorylation occurs despite the lack of significant changes in SR Ca^{2+} load (11) and the well-known decrease in I_{Ca} produced by HS (6, 20). This effect would counteract the negative inotropic action of HS, which would have been greater in the absence of RyR2 phosphorylation. Supporting this concept, Ho et al. (13) recently demonstrated that cholinergic stimulation of the heart produced an increase in SR Ca^{2+} release at low SR Ca^{2+} content compared with nonstimulated hearts. The consequent enhancement of FCaR, which occurs without alterations of I_{Ca}, is due to a facilitation of SR Ca^{2+} release produced by PKG-dependent phosphorylation of RyR2 at the Ser^{2808} site. The authors emphasized that this mechanism may be beneficial in failing hearts by preserving enhanced systolic release, counteracting, therefore, the heart failure-induced decrease in contractility. This type of result underscores again the importance of RyR2 P_o as an active regulator of cardiac function.

In this context, it is important to point out that more substancial increases in RyR2 P_o than that considered above may also occur. For instance, it has been shown that caffeine concentrations of ≥1 mM decrease Ca^{2+} transients (25). Also, in advanced heart failure, RyR2 has been described to be locked in a subconductance state (21), and enhanced RyR2-mediated Ca^{2+} leak has been shown to diminish intracellular Ca^{2+} transients (3). Moreover, reducing SR Ca^{2+} leak normalizes Ca^{2+} transient amplitude (15, 29). These results reveal that under these conditions, the expected Ca^{2+} flux balance that produces transitory effects on intracellular Ca^{2+} transients (12, 32) is not operative. Indeed, in overt heart failure, the severe decrease in SR Ca^{2+} would highly hamper the CICR mechanism (2, 5, 14). When in our model RyR2 conductance was increased to 100%, a decrease in the intracellular Ca^{2+} transient of ~20% was observed. Interestingly, even under these conditions, FCaR was still higher than control.

β-AR stimulation is also a particular case of an increase in RyR2 P_o that occurs associated with an increase in I_{Ca} and accelerated SERCA2a-mediated SR Ca^{2+} uptake. As shown in Fig. 2*C*, we simulated an increase in SR Ca^{2+} uptake of 30% and of I_{Ca} of 30%, which would mimic the effects of a low isoproterenol concentration, according to experimental data (7, 19) and a recently published model (23). As expected, there was a shift of the "control" point (*point A* in Fig. 2*C*) toward higher SR Ca^{2+} concentrations (*point B* in Fig. 2*C*). At this point, increasing RyR2 conductance by 15% and 25% transiently increased intracellular Ca^{2+} transients due to the increase in P_o, from *point B* to *point C* and *point D* in Fig. 2*C*, respectively. The Ca^{2+} transient then decreased (dashed arrow) according to the decrease in SR Ca^{2+} content, reaching values similar to *point B* (*points C′* and *D′*) but higher than *point A* (Fig. 2*C*), mimicking the positive inotropy of β-AR stimulation. Greater increases in RyR2 conductance, i.e, 50%, were associated in the model with the development of arrhythmias. This result indicates that the flux balance triggered by the opening of RyR2 (12, 31) also takes place under isoproterenol stimulation. Again, for a given SR Ca^{2+} load, the increase in RyR2 P_o results in an enhanced FCaR.

Finally, it is important to emphasize that the above analysis does not attempt to underestimate the negative influence of increasing RyR2 P_o on myocardial function due the enhancement of diastolic Ca^{2+} leak. In addition to the decrease in SR Ca^{2+} content, which necessarily affects Ca^{2+} transient amplitude, an increase in SR Ca^{2+} leak would increase diastolic Ca^{2+} and slow relaxation, leading to diastolic dysfunction and Ca^{2+}-triggered arrhythmias, as has been shown as a consequence of RyR2 mutations/phosphorylation and at different stages of heart failure (3, 4, 22, 33, 34). Indeed, experimental evidence indicates that the RyR2 stabilizer JTV519 reduces RyR2 P_o and protects nonfailing and terminally failing human myocardium from diastolic dysfunction induced by SR Ca^{2+} overload (24, 30). These unfavorable actions of the increase in SR Ca^{2+} leak

on cardiac function, which may prevail in the later stages of heart failure, would oppose and even eclipse the beneficial effects of RyR2 potentiation on systolic Ca^{2+} release. However, and as already discussed, these latter effects would contribute to either increase or maintain cardiac function under different conditions, including early stages of heart failure, despite enhanced diastolic Ca^{2+} leak (4).

In summary, the above considerations emphasize that the increase in P_o of RyR2 may be functional and would actively contribute to define a given inotropic state, counteracting their own detrimental effects on cardiac function.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

E.L., J.A.N., and A.M. analyzed data; J.A.N. performed model simulations; E.L. prepared figures; E.L., J.A.N., M.V.P., and A.M. edited and revised manuscript; E.L., J.A.N., M.V.P., and A.M. approved final version of manuscript; J.A.N., M.V.P., and A.M. interpreted results of experiments; A.M. conceived and designed research; A.M. drafted manuscript.

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