Treatment of Heart Failure Through Stabilization of the Cardiac Ryanodine Receptor

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The key event in cardiac excitation-contraction coupling is calcium-induced calcium release from the sarcoplasmic reticulum (SR) after activation of cardiac ryanodine receptors (RyRs). Two distinct genes encode the cardiac (RyR2) and skeletal muscle (RyR1) specific receptors. A third type of ryanodine receptor (RyR3) exhibits functional properties distinct from those of RyR1 and RyR2. The RyR channels form a tetrameric structure composed of 4 monomeric subunits, each of about 565,000 Daltons. These channels are about 10 times larger than the voltage-gated Ca$^{2+}$ and Na$^+$ channels. RyR2 opening increases cytosolic Ca$^{2+}$ from approximately 100 nmol/L to 1 μmol/L with consecutive activation of contractile proteins. The RyR was recently shown to be a macromolecular complex including protein kinase A (PKA) and its anchoring protein (mAKAP), the protein phosphatases PP1 and PP2A, sorcin, calmodulin, FK506 binding protein (FKBP), and other proteins. FKBP12.0 and its isoform FKBP12.6 seem to play an important role for the regulation of the RyR. The immunophosphoimunophen contributed to the cytosolic receptor for the immunosuppressant FK506 in T-cells. FKBP12 is a cis/trans peptidyl-prolyl isomerase. However, it is unlikely that this enzymatic activity is directly involved in RyR regulation. FKBP12 has been postulated to bind to the RyR with a stoichiometry of 4 or less FKBP12 molecules per single functional channel. FKBP12.0 is tightly bound to and regulates the function of the skeletal RyR1, whereas RyR2 has been found to co-purify selectively with FKBP12.6 despite a higher expression level of FKBP12.0 in cardiac myocytes. However, considerable species differences in binding preferences of FKBPs to RyR isoforms have been observed.

Marx et al$^9$ recently suggested that phosphorylation of RyR2 dissociates the regulatory protein FKBP12.6 from the channel, resulting in pronounced altered channel function. First, activity of the channel (open probability) is increased, which results from a leftward shift in the Ca$^{2+}$ dependence for channel activation. This increased sensitivity is associated with destabilization of the channel, resulting in subconductance states. Second, the dissociation of FKBP12.6 from the RyR functionally uncouples multiple RyRs and disturbs coupled gating. Coupled gating enables neighboring RyRs to open and close in a coordinated manner. Under uncoupling conditions, not all RyRs in a given region of the SR open together during systole and close together during diastole. Some RyRs remain open, resulting in a diastolic leak of Ca$^{2+}$ from the SR.$^{10}$

Marx et al$^9$ furthermore reported that in failing human hearts, the RyR is hyperphosphorylated, resulting in dissociation of FKBP12.6 from RyR. Accordingly, increased Ca$^{2+}$ sensitivity, subconductance states, uncoupled gating of RyRs, and, in consequence, SR Ca$^{2+}$ leak were described. Hyperphosphorylation may result from decreased levels of phosphatases (PP1 and PP2A) associated with the RyRs. Reduced FKBP12.6 to RyR binding in the failing heart was confirmed by Ono et al$^{11}$ using [H]$\text{dihydro-FK506}$–binding. They showed that in SER vesicles from dogs with pacing-induced heart failure, abundance of FKBP12.6 was decreased by 83% when compared with normal hearts.

The concept of hyperphosphorylation-induced RyR dysfunction in heart failure is still somewhat controversial. Recent studies in phospholamban knockout mice indicated that PKA-dependent RyR phosphorylation does not influence Ca$^{2+}$ leak and SR Ca$^{2+}$ release and that PKA-dependent effects on SR Ca$^{2+}$ release result from phospholamban phosphorylation, with subsequently increased SR Ca$^{2+}$. However, independent from the concept of RYR hyperphosphorylation in heart failure, there is considerable evidence that SR leak may be relevant for disturbed SR function in heart failure in addition to reduced SR Ca$^{2+}$ uptake, and FKBPs are important regulators for SR Ca$^{2+}$ handling. There is general agreement that disturbed SR function is the dominant mechanism that causes altered excitation-contraction coupling in human heart failure. It has been shown that frequency-dependent increase of SR Ca$^{2+}$ content, as it is observed in non-failing human myocardium, is blunted or absent in the failing heart, which results in lower SR Ca$^{2+}$ load and Ca$^{2+}$ availability for systolic release. Quantitatively, the contribution of Ca$^{2+}$ leak through RyRs to disturbed SR Ca$^{2+}$ accumulation is unknown, and altered SR Ca$^{2+}$ uptake may still be the major component. Ca$^{2+}$ uptake in heart failure is reduced because of decreased SR Ca$^{2+}$ ATPase activity due to decreased pump levels and reduced phospholamban phosphorylation. In addition, it has been suggested that increased Na$^-$–Ca$^{2+}$ exchanger activity may result in increased transsarcolemmal Ca$^{2+}$ elimination and SR Ca$^{2+}$ depletion. The relevance of FKBPs for SR Ca$^{2+}$ handling has been demonstrated in a recent study. It was shown that

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Marx et al$^9$ further investigated that phosphorylation of RyR2 dissociates the regulatory protein FKBP12.6 from the channel, resulting in pronounced altered channel function. First, activity of the channel (open probability) is increased, which results from a leftward shift in the Ca$^{2+}$ dependence for channel activation. This increased sensitivity is associated

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overexpression of FKBP12.6 in rabbit myocytes results in reduced spontaneous SR Ca\(^{2+}\) leak, increased SR Ca\(^{2+}\) content, and increased isotonic shortening of isolated myocytes. These findings confirm the postulated effects of FKBP12.6 to reduce Ca\(^{2+}\) leak from the SR through the RyR and promote Ca\(^{2+}\) release during activation.\(^\text{14}\)

Assuming that SR Ca\(^{2+}\) leak is relevant for the pathophysiology and progression of heart failure, restoration of RyR function could be an important target for the treatment of heart failure. A leak of Ca\(^{2+}\) from the SR decreases SR Ca\(^{2+}\) content and thus availability of systolic Ca\(^{2+}\) to be released; increased Ca\(^{2+}\) leak may promote diastolic dysfunction because of diastolic activation of contractile proteins; increased Ca\(^{2+}\) leak from the RyR may be a trigger for arrhythmias; altered cytosolic Ca\(^{2+}\) may contribute to altered gene expression and myocardial remodeling; and an increased leak would have unfavorable energetic consequences because ATP consumption for SR Ca\(^{2+}\) cycling would be increased for any given level of SR Ca\(^{2+}\) content. In this regard, reducing the leak seems to be advantageous over increasing SR Ca\(^{2+}\) uptake because increased SR Ca\(^{2+}\) content by itself increases Ca\(^{2+}\) leak in a nonlinear relation.\(^\text{15}\)

In this issue of Circulation, Yano et al\(^\text{16}\) reported that the agent JTV519, a 1,4-benzothiazepine, restores disturbed RyR function and preserves contractile performance in hearts from dogs with 4 weeks rapid right ventricular pacing-induced heart failure.

In this heart failure model, alterations of excitation-contraction coupling, as previously analyzed by Yano et al, are similar to those reported by Marx et al\(^\text{9}\) in humans, ie, increased contraction coupling, as previously analyzed by Yano et al, dogs with 4 weeks rapid right ventricular pacing-induced heart failure and preserves contractile performance in hearts from failing hearts. The data presented support the hypothesis. It is shown that JTV519 acts by exerting an FKBP12.6-like channel stabilizing effect either directly or by catalyzing re-association of FKBP12.6. The data presented support the hypothesis. It is shown that JTV519 reduces Ca\(^{2+}\) leak from SR vesicles of failing hearts. The drug is reported to restore binding of FKBP12.6 to RyR toward the stoichiometry seen in normal hearts. It decreases the effects of FK506 on FKBP12.6 dissociation from the RyR in a dose-dependent manner in immunoprecipitation experiments. Furthermore, it prevents cAMP-dependent reduction of FKBP-binding to RyRs. These effects were associated with preservation of FK506-induced Ca\(^{2+}\) leak in both normal and failing hearts.

The molecular mechanisms by which JTV519 may induce its actions are not completely understood. Obviously, there are no direct effects on RyR phosphorylation as shown by back-phosphorylation experiments. Interestingly, JTV519 as a benzothiazepine derivative, has structural similarities with the L-type Ca\(^{2+}\) channel blocker diltiazem and decreases L-type Ca\(^{2+}\) current by about 20%.\(^\text{17}\) Accordingly, diltiazem itself was found to partially inhibit SR Ca\(^{2+}\) leak. However, it can be excluded that the observed effect on SR leak results from L-type Ca\(^{2+}\) channel inhibition because measurements were predominantly performed in isolated vesicles. However, it cannot be excluded that part of the long-term effects on ventricular function (see below) may be due to L-type Ca\(^{2+}\) channel blockade or other reported effects of JTV519, such as influence on potassium currents\(^\text{19}\) or interaction with annexin V.\(^\text{19}\) On the other hand, the observed structural relationship to diltiazem may help to identify other molecules with even more specific effects to RyRs.

The chronic effects of intravenous JTV519 treatment are quite remarkable. When JTV519 was continuously infused by means of an infusion pump, it partially preserved systolic and diastolic function and prevented development of heart failure in chronically paced dogs. In addition, chronic administration of JTV519 reduced the levels of plasma norepinephrine, angiotensin II and atrial natriuretic peptide (ANP). Prevention of heart failure was associated with higher SR Ca\(^{2+}\)-ATPase levels, higher phospholamban phosphorylation, and thus increased SR Ca\(^{2+}\) uptake. In addition, PKA phosphorylation of RyR was lower compared with untreated pacing induced failing myocardium. The latter findings are most likely not primarily related to the effects of JTV519 on RyRs, and rather reflect prevention of left ventricular dysfunction and development of heart failure. Accordingly, in a recent article,\(^\text{20}\) propranolol treatment of dogs during tachycardia pacing prevented development of heart failure and of SR Ca\(^{2+}\) leak. This was associated with restoration of FKBP12.6 binding to RyRs. Furthermore, it was shown that β-blocker treatment reduced the level of PKA-dependent RyR phosphorylation and normalized single-channel RyR function in another study with pacing induced heart failure.\(^\text{21}\)

In summary, there is cumulative evidence that increased leak of Ca\(^{2+}\) through RyRs is a significant component of altered excitation-contraction coupling in heart failure. Accordingly, reducing SR leak is a promising approach to improve Ca\(^{2+}\) cycling of the failing heart that theoretically should be advantageous over strategies to increase SR Ca\(^{2+}\) uptake. The present study\(^\text{16}\) is the first to show the feasibility of pharmacologically improving RyR function and SR leak in isolated vesicles and to show that long-term application of this agent is associated with improved ventricular function and prevention of heart failure in a dog model. However, it cannot be excluded that a variety of other unspecific effects of this drug contributed to its beneficial effects in heart failure animals, in particular because the molecular mechanism of its effects on RyRs is not completely understood. However, the present work should stimulate research to better understand the effects of JVT519 on SR function and, even more importantly, to search for other interventions to reduce SR leak and improve Ca\(^{2+}\) cycling in heart failure.

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