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COMMENTARY

SERCA2a stimulation by istaroxime: a novel mechanism of action with translational implications

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Sarcoplasmic reticular (SR) Ca^{2+}-ATPase (SERCA2a) is central to cardiac electrophysiological and mechanical function. It ensures full diastolic relaxation minimizing delayed after-potentials that would otherwise compromise membrane electrophysiological stability, and optimizes SR Ca^{2+} refilling and systolic contraction. Previous studies demonstrated that the small molecule agent istaroxime stimulates SERCA2a-ATPase activity, restoring its function in failing hearts, and enhancing indices of mechanical, and SR Ca^{2+} release and re-uptake, activity. Ferrandi *et al* (2013) now elegantly demonstrate its ability to dissociate the phospholamdan (PB) bound to cardiac SERCA2a, thereby removing the inhibitory effect of PB on SERCA2a. This effect was independent of the cAMP/PKA system and modified a specific SERCA2a reaction step. They used SERCA-enriched SR preparations from a rigorously validated and realistic physiological, canine model of cardiac failure with established Na^+-K^+-ATPase sensitivity to cardiac glycosides and SR Ca^{2+} handling features. These findings potentially translate into a novel management of the major and increasingly important public health challenge of chronic cardiac failure.

LINKED ARTICLE

This article is a commentary on Ferrandi *et al.*, pp. 1849–1861 of volume 169 issue 8. To view this paper visit http://dx.doi.org/10.1111/bph.12278.

Abbreviations

CAMK, Ca^{2+}/calmodulin-dependent protein kinase; cAMP, cyclic adenosine monophosphate; CPVT, catecholaminergic polymorphic ventricular tachycardia; +dP/dt, left ventricular (LV) peak pressure generation; -dP/dt, LV peak pressure relaxation; $K_{d(Ca2+)}$, Ca^{2+} affinity constant; LV, left ventricle; PKA, protein kinase A; PLB, phospholamdan; RyR2, ryanodine receptor type 2; SERCA1, skeletal muscle sarcoplasmic reticular (SR) Ca^{2+}-ATPase; SERCA2a, cardiac SR Ca^{2+}-ATPase; SR, sarcoplasmic reticular; V_{max}, maximum reaction rate

Agents stimulating rather than inhibiting activity in specific biomolecules, particularly those involving novel mechanisms of action of both pharmacological and translational importance that thereby exert therapeutic effects on common and medically important conditions, merit positive editorial attention. The article by Ferrandi *et al.* (2013) in this volume decidedly fulfils such criteria. An elegant series of experiments characterizes the mechanisms of action by which the small molecule istaroxime stimulates cardiac sarcoplasmic reticular (SR) Ca^{2+}-ATPase (SERCA2a) activity. These findings were then related to possible translations into the management of the major and increasingly important public health challenge of cardiac failure (Khatibzadeh *et al.*, 2012).

SERCA2a is thought to have physiologically strategic and intricate roles in cardiac electrophysiological and mechanical function. Its diastolic translocation of released Ca^{2+} from cytosol to SR minimizes cytosolic [Ca^{2+}]. This ensures full myocardial relaxation, a normal ventricular diastolic compliance and filling, and minimizes delayed after-potentials that would otherwise compromise membrane electrophysiological stability. These functions thus prevent the diastolic dysfunction and arrhythmic tendency characteristic of cardiac failure. Recent reports from genetically modified *RyR2-P2328S* hearts have further implicated altered Ca^{2+} homeostasis in modulating Na^+ channel function and the resulting alterations in action potential conduction, which if slowed would produce arrhythmic substrate (King *et al.*, 2013). The

resulting maximization of the SR Ca^{2+} store in turn optimizes the systolic Ca^{2+} release essential for effective cardiac contraction. Abnormalities in both these diastolic and systolic aspects of ventricular function characterize acute cardiac failure (Bers, 2002).

A judicious and scholarly study (Ferrandi *et al.*, 2013) now completes a series of important papers on this novel agent. Together, these constitute a major contribution to this important cardiovascular field. The earlier papers had demonstrated that istaroxime stimulates SERCA2a-ATPase activity in both healthy and failing guinea pig and human SR cardiac preparations, restoring SERCA2a activity in failing hearts to near-normal levels (Micheletti *et al.*, 2007). These findings accompanied enhancements in both twitch amplitude and relaxation consistent with increased systolic Ca^{2+} transients and accelerated SERCA2a-mediated diastolic Ca^{2+} SR reuptake in guinea pig (Rocchetti *et al.*, 2005) and mouse isolated cardiac myocytes (Alemanni *et al.*, 2011). These actions contrasted with its inhibition of activity in canine kidney purified Na^{+}-K^{+} ATPase preparations.

An exemplary multidimensional approach employed a wide range of techniques with nevertheless corresponding and comparable outcomes. The experiments utilized SERCA-enriched SR preparations from a rigorously validated and realistic physiological canine cardiac platform with established Na^{+}-K^{+}-ATPase sensitivity to cardiac glycosides and SR Ca^{2+} handling features clinically translatable to humans (Sabbah *et al.*, 1991), for which istaroxime is therapeutically effective in chronic cardiac failure (Adamson *et al.*, 2003; Mattera *et al.*, 2007; Sabbah *et al.*, 2007). The presence of cardiac failure was established by objective physiological criteria of reduced left ventricular (LV) ejection fraction, increased LV end-diastolic pressures and volumes, decreased cardiac output, reduced peak LV pressure generation (+dP/dt) and relaxation (-dP/dt) and increased pulmonary artery wedge pressures. Results were matched with those obtained using a rabbit skeletal muscle SERCA1 as opposed to the cardiac muscle SERCA2 preparation for comparison with a system lacking the phospholamdan (PLB)-mediated inhibition mechanism found with cardiac SERCA2a. The experiments were complemented by *in vitro* studies in Sf21 cells variously expressing canine SERCA2a and PLB, and SERCA1 to examine contributions from SERCA2a-PLB interactions.

The measurements in the SERCA-enriched preparations permitted assessments not only of the cyclopiazonic acid sensitive Ca^{2+}-ATPase activity itself, but also of the steady state, and the initial fast phase of SERCA-mediated SR $^{45}Ca^{2+}$ uptake. The latter were compared with direct bilayer ATP-induced charge movement assessments of SERCA2a-mediated Ca^{2+} transfers immediately following ATP utilization (Tadini-Buoninsegni *et al.*, 2010). Charge movement measurements have been strategic in directly assessing charge transfers, membrane protein configurational changes and their associated interactions in a wide range of situations elsewhere, particularly in ion channel biophysics and excitation-contraction coupling (Huang *et al.*, 2011). Results of these dynamic studies could then be correlated with the results of more routine co-immunoprecipitation and Western blot assays for PLB expression.

Reductions in maximum reaction rates, V_{max}, and affinity constants, $K_{d(Ca2+)}$, in SERCA2a-ATPase activity were confirmed in failing compared to healthy hearts and related to reduced (~20%) SERCA protein and monomeric (~21%) PLB expression. Istaroxime, used in these studies at concentrations between 0.0001 and 100 nM, then increased such activity, doing so at lower concentrations (1 nM) and, therefore, probably with a higher potency in failing than in healthy heart SR vesicles (100 nM). Istaroxime similarly increased $^{45}Ca^{2+}$ uptake into cardiac SR vesicles, as reflected in measurements of both steady state V_{max}, and transients obtained from stopped flow measurements, consistent with previous findings in guinea pig and human preparations (Rocchetti *et al.*, 2005; Micheletti *et al.*, 2007). Functional measurements correspondingly demonstrated increased peak Ca^{2+}-dependent charge movement associated with the SERCA2a E2 to E1 transition following ATP jumps, in cardiac SERCA2a but not skeletal muscle SERCA1 preparations. The latter findings were compatible with suggestions that istaroxime acts by displacing PLB from the SERCA2a/PLB complex, thereby removing the inhibitory action of PLB on this complex. This mechanism is also implicated in the physiological actions of either PKA or Ca^{2+}/calmodulin-dependent protein kinase (CAMK) through PLB phosphorylation at Ser^{16} and Thr^{17} respectively (Traaseth *et al.*, 2006; Bidwell *et al.*, 2011). Istaroxime similarly increased the V_{max} of Ca^{2+} transport in microsomes from Sf21 cells over-expressing both cardiac SERCA2a and PLB, but not SERCA2a or SERCA1 alone. This action was independent of the addition of the PKA inhibitor staurosporin; thus, it is unlikely cAMP/PKA-mediated mechanisms are involved in this effect of istaroxime. In contrast, istaroxime reduced the co-immunoprecipitation of SERCA2a with PLB at 0.1, but not at 1 and 5 μM Ca^{2+}, suggesting it disrupted the physical interaction between them.

Taken together, these findings demonstrate a novel pharmacological action of istaroxime in dissociating the SERCA2a-PLB complex through mechanisms independent of the cAMP/PKA system thereby removing the inhibitory effect of PLB binding. The resulting modification of the SERCA2a E2 to E1 transition then accelerates Ca^{2+} cycling. This would have translational implications through the resulting, positive, effects upon the cardiac contraction-relaxation cycle particularly in failing hearts (Gheorghiade *et al.*, 2008; Shah *et al.*, 2009). To this end, strategies involving cAMP/PKA signalling might contribute to the management of chronic cardiac negative remodelling and failure. Agents promoting PLB phosphorylation such as isoprenaline and phosphodiesterase inhibitors could acutely increase cardiac contractility in cardiac failure, although questions remain concerning long-term effects (Cuffe *et al.*, 2002). Alternative gene transfer strategies directed at SERCA2a entail issues concerning their clinical application (Del Monte *et al.*, 1999). Within this setting, this new agent for improving cardiac function with novel and complementary mechanisms of action certainly merits further investigation and testing in both the laboratory and the clinic. It also opens up possibilities for additional therapeutic explorations directed at arrhythmic conditions whether accompanying cardiac failure (Rocchetti *et al.*, 2003) or occurring in their own right. For the latter case, the genetic condition of catecholaminergic polymorphic ventricular tachycardia, with its implications for SR Ca^{2+} resulting from increased RyR2-mediated Ca^{2+} release or altered SR calsequestrin binding, might offer a characterizable example (e.g. King *et al.*, 2013).

Conflict of interest

None declared.

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