Return of calcium: Manipulating intracellular calcium to prevent cardiac pathologies

Yibin Wang*†‡ and Joshua I. Goldhaber†‡

*Departments of *Anesthesiology and †Medicine, Cardiovascular Research Laboratories, David Geffen School of Medicine, University of California, Los Angeles, CA 90095*

Heart failure resulting from ischemia/reperfusion and other forms of injury is characterized by a variety of pathological manifestations, including cellular hypertrophy, contractile dysfunction, and electrical instability. Abnormal calcium signaling leading to cytoplasmic calcium overload is thought to be a critical and perhaps common mechanism underlying these abnormalities. Since the 1980s, it has been known that inhibition of cardiac metabolism leads to increased intracellular calcium (1), and that pharmacologic therapies aimed at blocking calcium entry into cells not only reduce cellular injury (2), but also decrease the frequency of ventricular arrhythmias (3). More recently, studies using targeted genetic approaches have demonstrated that manipulating cardiomyocyte calcium handling can prevent or reduce the progression of hypertrophy and cardiac dysfunction associated with aging, ischemia, reperfusion, and pressure overload [see Sobie *et al.* (4) for review] but have also raised concerns about the potential for worsening heart failure (5, 6). In a recent issue of PNAS, del Monte *et al.* (7) use a genetic strategy to modify cellular calcium handling during ischemia by overexpressing sarcoplasmic reticulum ATPase via an adenovirus vector. Similar to pharmacologic strategies for reducing cytosolic free calcium, such as calcium channel blockers and beta-blockers, SERCA overexpression not only reduces infarct size and preserves cardiac function, but also reduces arrhythmia frequency. The success of this approach once again supports the long-held notion that calcium cycling is an important therapeutic target to prevent the deleterious consequences of ischemia/reperfusion injury.

Calcium Cycling and Normal Cardiac Function

Carefully regulated calcium cycling is critical for cardiac function, which depends on the calcium concentration surrounding the myofilaments rising and falling in a cyclic manner in response to membrane depolarization. Insufficient calcium delivery to the myofilaments results in a weak contraction, whereas excessive calcium delivery carries the risk of contracture, activation of proteases and other maladaptive calcium-sensitive pathways that lead to cell death, and generation of pathological membrane currents. A variety of ion channels, ATP-dependent pumps, and transporter proteins serve as the major control points of calcium regulation in the heart, including L-type calcium channels and ryanodine receptors (RyR) for calcium entry and release from SR, as well as SERCA and sodium–calcium exchanger (NCX) for calcium uptake or extrusion (see Fig. 1 for details) (8–10).

Calcium-Mediated Arrhythmias

The role of abnormal calcium signaling in the genesis of cardiac arrhythmias has generated considerable interest over the past three decades [for review, see Clusin (11)]. The classic calcium-mediated arrhythmia is produced by excessive doses of cardiac glycosides such as digitalis. These agents work by raising intracellular sodium, which in turn reduces calcium efflux by the NCX, and thus favors net calcium uptake by the SR (12). At toxic doses, cardiac glycosides produce calcium overload of the SR, which results in spontaneous release of calcium by RyRs, thereby generating a depolarizing inward current mediated by NCX (which is electrogenic, exchanging three Na^+ ions for one Ca^{2+} ion) (13). These spontaneous events are known as delayed afterdepolarizations (DADs), and they underlie so-called triggered arrhythmias seen in some heart failure models (14, 15). NCX up-regulation during heart failure further increases the likelihood of effective depolarization by spontaneous SR calcium release (15). DADs may also be generated under conditions of abnormal diastolic calcium leak through hyperphosphorylated RyRs, such as may occur in the setting of heart failure (16) or in rare familial arrhythmias associated with RyR missense mutations (17). Early afterdepolarizations (EADs) are another source of arrhythmias, caused by prolonged action potentials (due to failure of inward Na^+ currents to inactivate or failure of outward K^+ currents to activate) caused by drugs or mutation-induced dysfunction of Na^+ or K^+ channels (18), thus allowing excessive calcium entry through L-type calcium channels (19, 20). Finally, abnormal calcium cycling by the SR has been implicated in the pathogenesis of action potential alternans (21) and spiral wave break-up leading to the development of ventricular fibrillation (22). SERCA dysfunction is a common finding in heart failure (23). Thus, all aspects of calcium cycling are inviting targets for antiarrhythmic strategies.

Overexpressing SERCA dramatically alters the balance between the major calcium-handling proteins. Like digoxin, SERCA overexpression favors sequestration of calcium by the SR instead of being extruded by the NCX. What are the implications of this larger SR store for E–C coupling and arrhythmogenesis? Eisner *et al.* (24) have pointed out that although a larger SR store will initially lead to an increase in the calcium transient, autoregulation is ensured by (*i*) more rapid inactivation of subsequent calcium currents and, therefore, (*ii*) reduced calcium entry through L-type calcium channels. The net effect is to reduce transarcolemmal calcium flux while maintaining a normal systolic transient. Thus, one might expect SERCA overexpression to reduce the L-type current, recapitulating the effects of calcium channel blockade on arrhythmia prevention. Further experiments will be required to test this hypothesis.

Is it advantageous to genetically increase SERCA activity rather than directly blocking the L-type calcium channel? One potential advantage is the preservation of blood pressure, a key limitation to the clinical utility of calcium channel blockers, which are not unequivocally beneficial in humans with ischemic syndromes (11). Another advantage of SERCA overexpression is superior relaxation kinetics of the calcium transient, which protects the diastolic filling period and thus limits the duration of exposure of cytosolic components to elevated calcium. It is also possible that increased inactivation of the L-type calcium current in the setting of the increased SR calcium load due to SERCA overexpression may reduce the likelihood of action potential alternans, and thus reduce the propensity for reentry. The potential benefits of improving SR calcium uptake are also supported by a string of recent successes targeting the same machinery. Inactivation

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‡To whom correspondence may be addressed. E-mail: yibinwang@mednet.ucla.edu or jgoldhaber@mednet.ucla.edu.

of phospholamban (PLB), an endogenous inhibitor of SERCA, has been shown to prevent or "rescue" the pathological phenotype in some (25–28), but not all, models of heart failure (6).

Risks of SERCA Overexpression

Despite the significant promises brought by the success of the gene therapy approach described in this report, it is wise to take a cautionary note when we attempt to translate the observation made in rodents into human clinics. Significant differences do exist between them in terms of the regulatory mechanisms of calcium cycling, the relative contribution of SR calcium vs. L-type calcium channel mediated calcium entry to total intracellular calcium, and the triggering factors for ventricular arrhythmia. These differences make it difficult to predict the outcome of SR calcium manipulation perceived from small animal models. Indeed, PLB inactivation, although apparently beneficial in rodent models of heart failure, appears to be deleterious in humans with a familial form of heart failure characterized by defective PLB (5). There are also potential inherent risks to SERCA overexpression, particularly the risk of SR calcium overload. SR calcium overload, as in the case of digitalis toxicity, is clearly proarrhythmic. In addition, when using genetic approaches to arrhythmia management one must always beware of introducing heterogeneity into the myocardium as a result of somatic gene transfer, adding another potential cause of electrical instability leading to fibrillation (29). Because of the limited duration of gene expression via Adv mediated gene transfer, the long-term effects of SERCA overexpression on survival and remodeling cannot be fully assessed. A better gene delivery system with efficient and prolonged expression is needed, perhaps the adeno-associate virus demonstrated in a recent study by Iwanaga *et al.* (27).

Clearly, the study reported here raised many interesting questions regarding cellular consequences of shifting the balance of calcium cycle in SERCA overexpressed cells and its effects on other stress-activated pathways related to ischemia/reperfusion, apoptosis, and remodeling. Nevertheless, we can view this as yet another motivation for us to return to the fundamental mechanism of heart failure and arrhythmia in search for better therapeutic approaches.

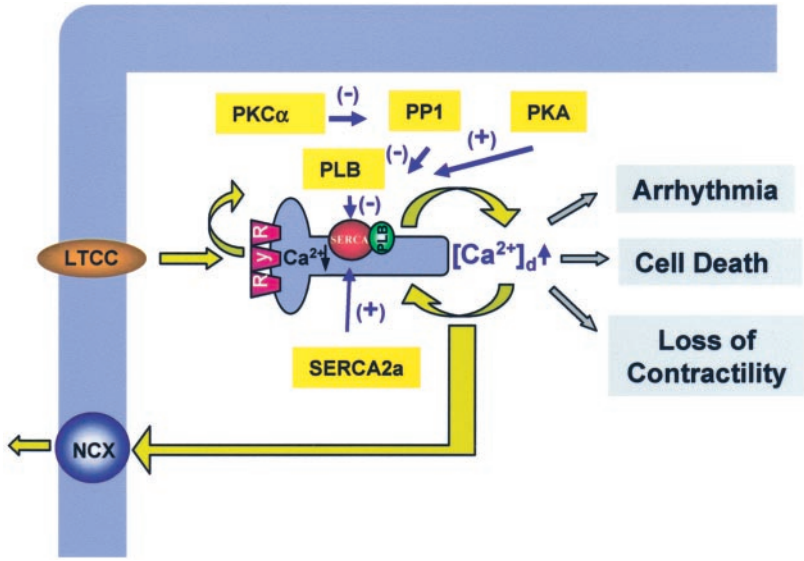

Fig. 1. Major control points for calcium in cardiac myocytes. A small amount of calcium enters cells through the L-type calcium channel (LTCC), triggering release of a much larger amount of calcium from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). Most of the calcium is pumped back into the SR by SERCA2a, and the rest is extruded from the cell by the NCX. Calcium uptake by SERCA is regulated by the inhibitory protein phospholamban (PLB), which is dependent on signaling pathways involving PKCα, PP1, and PKA. At the end of the cardiac cycle, calcium efflux must balance calcium influx. del Monte *et al.* (7) show that in the setting of ischemia/reperfusion injury, overexpression of SERCA2a hastens removal of calcium from the cytosol and reduces cell death, contractile dysfunction, and arrhythmias.

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