Mechanisms and Use of Calcium-Sensitizing Agents in the Failing Heart

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Depressed cardiac contractility is central to many forms of cardiac disease and reflects the heart’s inability to generate adequate force despite being provided physiological activator calcium and chamber load. Yet, successful methods to enhance cardiac contractility have remained elusive. Agents such as dobutamine or milrinone that work through activator calcium and chamber load. Yet, successful methods contributed to their vasodilation/venodilation and Ca2+ inhibiting cAMP phosphodiesterase (PDE3a), that likely force. The class of molecules that achieve this are often by which intracellular calcium is transduced into muscle such complications would be to directly influence the manner making hearts less efficient. An alternative approach to avoid such effects are achieved vary widely and include direct activators of motor proteins such as myosin, enhancers of force generated by a cross-bridge, and agents that augment Ca2+-troponin C (TnC) binding and its consequences. To date, many such drugs have had additional effects, such as inhibiting cAMP phosphodiesterase (PDE3a), that likely contributed to their vasodilatation/venodilation and Ca2+-dependent increases in heart rate and contractility. Other agents, such as levosimendan, also inhibit ATP-sensitive potassium channels, which can induce further effects.

This review discusses basic molecular mechanisms for drugs that alter the myofilament response to calcium, how such agents affect muscle and whole-organ physiology, and what their clinical testing has revealed. In doing so, we attempted to bridge the gap between existing reductionist reviews to those that have focused largely on clinical outcome trials.

Excitation/Contraction Coupling

Physiological and pharmacological modifications in myofilament response to Ca2+ occur as an integral mechanism of excitation/contraction coupling. In diastole, Ca2+ concentrations surrounding the sarcomere of working heart muscle cells fall below the myofilament activation threshold. With electrical stimulation, Ca2+ moves into the cytoplasmic space and binds to the myofilaments, inducing force and contraction that are ~20% to 25% of maximum. One way to increase contractile force is to increase the Ca2+ that enters the myofilament space, and this is physiologically achieved by an elaborate set of membrane-related mechanisms that control Ca2+ fluxes. Alternatively, one can regulate how the sarcomere responds to Ca2+. Normally, changes in sarcomere length and the phosphorylation of regulatory proteins associated with thin and thick filaments modify Ca2+ sensitivity and rates of filament sliding, thereby modifying contractile reserve in concert with Ca2+ flux to and from the sarcomeres. Pharmacological approaches that further enhance the sarcomeric Ca2+ response modify this normal control mechanism.

The Myofilament Force-Ca2+ Relationship

A common approach to assessing myofilament Ca2+ activation is to apply varying Ca2+ concentrations (~10^-9 to 10^-4 mol/L) to single cells or muscle fibers in which the membranes have been removed by detergent. Muscle or sarcomere length is held constant, and developed tension is then measured at each Ca2+ level. Alternatively, intact trabeculae can be studied by chemically inactivating intracellular Ca2+ fluxes, opening sarcoplemmal Ca2+ channels, and tetanically stimulating the muscle while varying extracellular Ca2+ concentration. The resulting steady-state relation between Ca2+ and tension is sigmoidal in shape (Figure 1) and, once fit to the Hill equation, provides several parameters: The half-maximal activating Ca2+ concentration (Ca2+ sensitivity), steepness of the relation, and maximum tension. Each parameter varies physiologically and pathophysiologically and can be altered pharmacologically.

Figure 1A demonstrates the types of changes that can occur in the force-Ca2+ relationship that reflect myofilament responsiveness to Ca2+. One is a pure leftward shift of the relation without alterations in maximal (or minimal) Ca2+-activated force (type A). Such shifts occur from a decline in protein kinase A (PKA) phosphorylation of troponin I (TnI),...
or myocyte alkalization. This type of change would primarily manifest in normal operating ranges of Ca\(^{2+}\) activation. A potential problem is that force generation is also greater at diastolic calcium levels, which could increase passive stiffness. A second type of change includes a rise in the maximal activated force (type B). Changes of this nature are observed if the cross-bridge force generation is itself altered, as has been observed with changes in redox state, with proteolytic cleavage of TnI in postischemic stunned myocardium, and with sensitizers such as EMD-50733.6 The extent to which cleavage of TnI in postischemic stunned myocardium, and been observed with changes in redox state, with proteolytic cleavage of TnI in postischemic stunned myocardium, and with sensitizers such as EMD-50733.6 The extent to which such upward shifts occur at low calcium levels might again exacerbate diastolic effects. Lastly, a sensitizer may display Ca\(^{2+}\)-dependent changes, so that effects at lower calcium concentrations are minimized, whereas shifts in the force-Ca\(^{2+}\) relation at systolic levels of calcium are manifest (type C). Levosimendan is an example of an agent thought to have this property.7 The force-Ca\(^{2+}\) relation can also reflect simply a rise in maximal activated force but with no actual change in calcium sensitization (type D). In this case, normalization of each relation to maximal force results in superimposable curves.

In intact muscle, an increase in force by means of Ca\(^{2+}\) sensitization appears functionally quite different from that due to activation of cAMP-PKA–dependent mechanisms (Figure 1B). In the case of the latter, inotropy is coupled to shortening of the twitch duration and enhanced relaxation rate, due to faster E-C coupling kinetics from PKA phosphorylation of calcium handling and sarcomeric proteins (eg, voltage-gated Ca\(^{2+}\) channel, TnI, phospholamban, and myosin-binding protein C [MyBP-C]). In contrast, a Ca\(^{2+}\) sensitizer usually results in a similar rate of tension rise and net prolongation of systole. Relaxation can be slower or achieved at rates similar to those under basal conditions. At faster heart rates, these differences can become important, where diastolic time period and thus filling could be compromised. Most Ca\(^{2+}\)-sensitizer agents have combined primary with secondary pharmacological effects, the latter often offsetting some changes that might arise from a pure sensitization response. An example is levosimendan, which combines myofilament modifications with activation of the ATP-sensitive potassium channel and inhibition of phosphodiesterase 3A (PDE3a).

### Molecular Mechanisms of Actin-Myosin Cross-Bridge Activation

A rational starting point for how one might use drugs to modify the mechanical activity of cardiac sarcomeres is to consider what regulates the reaction between myosin molecular motors (cross-bridges) of thick filaments with thin-filament actin. Figure 2 illustrates this understanding in a cartoon of the working (A band) region. During diastole, active tension is essentially zero and the myosin ATPase rate low. Depending on the sarcomere length, there is a passive tension dependent largely on stress-strain relations of the giant protein, titin.8 Cross-bridges are blocked from reacting with actin by the position of tropomyosin (Tm) on the thin filament, which covers the myosin-binding sites on the thin filament and alters the reactivity of actin with myosin. The diastolic state requires the binding of the tropomin complex (cTn) to the actin-Tm thin-filament backbone (Figure 2). Troponin is a complex of the following 3 proteins: cTnC, a protein that senses and binds Ca\(^{2+}\); cTnI, an actin-binding protein named for its ability to inhibit the actin-myosin interaction; and cTnT, named for its ability to bind Tm. cTnC is a dumbbell-shaped protein with a single regulatory Ca\(^{2+}\)-binding site at its N-terminal lobe and 2 structural Ca\(^{2+}\) binding sites at its C-terminal lobe (Figure 3). The lobes are connected by a linker helix. The N-lobe of cTnC contains a ring of amino acids that coordinate binding of a single Ca\(^{2+}\) with high affinity and fast kinetics. Without Ca\(^{2+}\) bound in the ring, a sticky patch made of hydrophobic side arms of amino acids remains concealed in the structure and is unavailable to interact with its neighbor, cTnI. In diastole, cTnI is kept away from the regulatory site of cTnC by multiple protein-protein interactions among troponin components and actin-Tm that serve to anchor the troponin complex to actin and restrict Tm...
movement. As shown (Figure 3), cTnC is held in place by a network of protein-protein interactions of its C-lobe with both cTnI and cTnT. The interactions consist of hydrophobic interactions, charge-charge interactions, and intertwining of helixes. The versatility in the interactions allows for versatility in control and modification. In diastole, Tm (tropomyosin) blocks the cross-bridge formation. In the absence of Ca$^{2+}$ bound to the N-lobe of TnC, Tm is held in this position by protein-protein interactions that involve Tnl, which tethers troponin to actin, and by TnT, which interacts with actin-Tm. In diastole, the N-lobe of TnC does not interact with the C-terminal region of Tnl, whereas with Ca$^{2+}$-TnC interaction, these regions bind triggering movement of Tm through the action of TnT. The cross-bridge is further modulated by interactions with myosin light chains (MLC) and MyBP-C, which also interacts with TnI, a molecular spring protein in the sarcomere.

Ca$^{2+}$ binding to cTnC triggers systole by inducing movements of the thin-filament proteins so that blocked cross-bridges may react with actin in a cycle of interactions that impel the thin filaments on each side of the sarcomere toward the center (Figure 2). A key aspect of the molecular mechanism that triggers contraction is a Ca$^{2+}$-induced hydrophobic cleft or sticky patch on the N-lobe of cTnC.$^9,10$ The hydrophobic switch peptide of cTnI is also sticky and binds to the cTnC hydrophobic cleft and sets into motion a reversal of the diastolic state. Key steps include the release of the actin-binding regions of cTnI from actin, and most likely an altered interaction of the cTnT N-terminal tail with Tm. One of the striking features of cardiac myofilaments is that the relation between Ca$^{2+}$ and tension is steep (Figure 1A), which indicates an underlying cooperative process.$^{11}$ What this means is the interaction of a cross-bridge with the thin filament makes it easier for a neighboring cross-bridge to bind. This is accomplished by an influence of cross-bridge binding on the position of Tm on the thin filament. The Tm are connected end-to-end to form a continuous strand; when a cross-bridge binds the Tm strand, movement affects the reactivity of a neighboring cross-bridge for actin. With this basic review as a foundation, we turn next to how this interaction can be modified by targeted pharmacological interventions.

**Thin Filament Proteins as Potential Drug Targets**

**Targeting the cTnC-cTnl Interaction**

There are 4 main interaction sites between cTnC and cTnl that are potential targets for inotropic drugs. One is the interaction of the sticky patch in the N-lobe of cTnC with the switch peptide of cTnl. An attractive aspect of this site is that the opening of the sticky patch in heart muscle by Ca$^{2+}$ is fundamentally different from that of fast skeletal TnC. An example of a drug that binds to this region is bepridil, which was not developed as a Ca$^{2+}$ sensitizer yet was found to bind to cTnl.$^{12}$ Structural studies showed that bepridil binds to the N-lobe of cTnC and induces an open configuration similar to that induced by Ca$^{2+}$ in the cTnC-cTnl complex.$^{13,14}$ The docking site of bepridil is different from the docking site of the switch peptide of cTnl, and thus both the drug and the switch peptide could bind to the cTnC N-lobe. Li et al.$^{13}$
hypothesized that bepridil may sensitize the myofilament to Ca\(^{2+}\) by slowing down both the closure of the hydrophobic cleft and the dissociation of cTnI on release of Ca\(^{2+}\) from site II of cTnC.

Another point of interaction of cTnC with cTnI is at a near N-terminal peptide of cTnI that binds tightly to a hydrophobic region of the cTnC C-lobe. Activation of the myofilaments may be altered by phosphorylation of the cTnI peptide, which indicates that this region is important in transduction of the Ca\(^{2+}\) binding signal at the N-lobe.\(^\text{15}\) A Ca\(^{2+}\)-sensitizing drug demonstrated to bind at this interface is the thiaazinone EMD-53998, which is a racemic mixture of the enantiomers (+)EMD-57033 and (−)EMD-57439. The racemic EMD-53998 increases Ca\(^{2+}\) sensitivity of myofilament force and ATPase rate but also is a phosphodiesterase inhibitor. Ca\(^{2+}\)-sensitization is a property of EMD-57033, and phosphodiesterase E (PD) inhibition is a property of EMD-57439.\(^\text{16}\) This evidence supported the idea of a receptor domain, which was subsequently identified as the C-lobe of cTnC.\(^\text{17}\) Determination of the nuclear magnetic resonance structure of cTnC demonstrated that EMD-57033 docks with its chiral group situated deep in the hydrophobic cleft in the cTnC C-lobe.\(^\text{18}\) This special localization suggests a structural basis for the lack of effects of the (−)-enantiomer on myofilament response to Ca\(^{2+}\). Drug binding competes with binding of cTnI in this region that EMD-57033 was completely displaced by cTnI(34-71) but not by a region that contained the cTnI switch peptide.

A third interface important in the action of Ca\(^{2+}\) sensitizers is between the actin-binding inhibitory peptide of cTnI and the helix that connects the N- and C-lobes of cTnC. The Ca\(^{2+}\)-sensitizing drug lesvosimendan (Simdax), a pyridazinone-dinitrile derivative, which was screened explicitly for binding to cTnC, docks at or near this region and is likely to alter the lifetime of the active state of the thin filament.\(^\text{19}\)

A fourth site of interaction of cTnI with cTnC is between the unique N-terminal peptide of cTnI and the N-lobe of cTnC (Figure 3). With the peptide bound, the affinity of TnC for Ca\(^{2+}\) is rather high; when the peptide is not bound, the affinity is lower.\(^\text{3}\) Release of the peptide from the N-lobe of cTnC occurs with phosphorylation of the cTnI N-terminal peptide associated with adrenergic stimulation. This interaction is modified physiologically and is important in regulation of the cycle time of the heart beat and in muscle shortening against an afterload. Drug-binding sites at the N-terminus have not been discovered or studied, but interactions of the N-terminal peptide of cTnI with cTnC have been reported to alter the Ca\(^{2+}\)-sensitizing effects of lesvosimendan.\(^\text{20}\)

**Targeting cTnI-Actin Interactions**

In general, there have been no attempts to alter the 2 regions of interaction of cTnI with actin by drug binding, although these regions seem especially important to consider. One region is the inhibitory peptide of cTnI, which binds to actin in diastole and is released in systole. In cTnI, this region contains a proline in skeletal TnI. Phosphorylation at this site alters myofilament response to Ca\(^{2+}\) and filament sliding velocity.\(^\text{15}\) A second binding site has been identified as critical through studies of myofilament and cardiac function in transgenic mice (ssTnI-TG) in which slow skeletal TnI (ssTnI) completely replaced cTnI. Compared with controls, ssTnI-TG myofilaments are more sensitive to Ca\(^{2+}\) and thus provide an animal model with myofilaments that are constitutively sensitized to Ca\(^{2+}\). Hearts of these mice demonstrate a remarkable resistance to acidosis,\(^\text{21}\) sepsis,\(^\text{22}\) and ischemia-reperfusion injury.\(^\text{23}\) There is evidence that a difference in a single amino acid (His/Ala) in ssTnI versus cTnI at position 162 is responsible for these functional differences.\(^\text{24}\)

**Tropinin T as a Target**

A particularly sensitive region of cTnT is in a domain in the C-terminus that surrounds amino acid 206.\(^\text{15}\) Modification of this region either by phosphorylation or by mutations linked to cardiomyopathies induces significant changes in tension and shortening of muscle. This region appears to function importantly in the action of cTnT as a lever in its role of moving Tm away from the actin sites that react with cross-bridges. The N-terminal tail of cTnT is also important in the transition from the diastolic to the systolic state, and thus, modification of this interaction could prove a useful drug target. The position of the region away from the core of troponin, as illustrated in Figure 3, also indicates that multiple protein-protein interactions might not be altered, as is the case with a drug that binds to the core structure.

**Thick-Filament Proteins as Potential Drug Targets**

The actin-myosin interface is also a potential site of action for Ca\(^{2+}\)-sensitizing drugs, although screening for agents that interact with myosin has not been a general approach. For example, EMD-57033 is able to increase the Ca\(^{2+}\)-independent actin-myosin interaction in preparations that lack Tn-Tm.\(^\text{16}\) There are also accessory proteins in thick-filament proteins that modify the actin–cross-bridge reaction but that have not been explicitly investigated as targets for Ca\(^{2+}\)-sensitizing agents. These include the essential (MLC1) and the regulatory (MLC2) light chains of myosin and MyBP-C (Figure 3). The light chains of myosin are an important determinant of myofilament sensitivity to Ca\(^{2+}\) and the kinetics of the reaction of cross-bridges with actin by providing structural stability to the cross-bridge and also by determining the extent of radial movement of cross-bridges away from the thick-filament backbone.\(^\text{4}\) This radial movement is apparently determined by the state of phosphorylation of MLC2. Variations in phosphorylation of the MLC2 in various regions of the heart appear important in coordinating cardiac torsion in ejection, MyBP-C, which serves both structural and regulatory functions, is part of a protein network that involves the myosin rod, the myosin head, and titin (Figure 3).\(^\text{25}\) The cardiac isoform is unique in possessing sites phosphorylated in response to altered adrenergic signaling. Studies with mouse hearts lacking MyBP-C indicate that it is critical for the maintenance of power and of muscle stiffness during systolic ejection.\(^\text{26}\) Thus, both MLC2 and MyBP-C regulate cross-bridge kinetics, and modifications of their protein-protein interactions may be a route to improve the power of contraction.
Ca\textsuperscript{2+} Sensitization and Agents That Affect Kinases and Phosphatases

There are a multitude of kinases that phosphorylate sarcomeric proteins and a growing number of phosphatases and phosphatase regulators that reverse this process, and these are also targets for modifying the myofilament response to Ca\textsuperscript{2+}. This approach has not yet been developed specifically for inotropic enhancement, and its full discussion falls outside the scope of this review. However, an example will serve to highlight this growing area of research. LY-379196, an inhibitor of protein kinase C-\beta (PKC-\beta), is an agent developed by Lilly with potential applications to cancer and diabetes. PKC-\betaII expression increases in human heart failure and can phosphorylate cTnT and cTnI, leading to a depressed myofilament response to Ca\textsuperscript{2+}. Thus, inhibition of PKC-\betaII might be expected to enhance Ca\textsuperscript{2+} sensitization, and this has been confirmed in animal models. The major challenge with this approach is specificity of action, which requires identification of key steps in the signaling pathway that leads to activation of phosphatases and kinases.

Impact of Calcium Sensitizers on the Intact Heart

Figure 4 summarizes several of the key features of calcium sensitizers in the intact heart, using EMD-57033 by way of example. One feature is the ability of the sensitizers to enhance contractility similarly in normal or failing hearts, in striking contrast to \beta-adrenergic stimulation (Figure 4A).
Because the target of Ca$^{2+}$ sensitizers is distal to PKA-mediated signaling, they display minimal downregulation in the failure state. Kinetic-dependent parameters such as dP/dt$_{max}$ are less affected by EMD-57033 than β-agonists, whereas late-systolic parameters that would more likely reflect enhanced cross-bridge force, such as end-systolic elastance, are similarly enhanced in controls (Figure 4B). In failure hearts, the response of both dP/dt$_{max}$ and Ees to dobutamine are depressed, whereas Ees increases with EMD-57033 much as in control hearts. This supports the targeting of mechanisms distal to the down-regulated β-adrenergic cascade.

Calcium sensitizers have variable effects on diastolic function depending on their mechanism(s) of action and the type of preparation studied. For example, at lower doses, EMD-57033, acting as a sensitizer with minimal PDE3 inhibitory effects, increases diastolic pressures and delayed relaxation in isometric cardiac muscle or isovolumic isolated hearts. However, in ejecting hearts, the same agent did not impair diastolic function. Sensitizers themselves are unlikely to enhance the rate of pressure relaxation, yet they can enhance diastolic filling rates (Figure 4C). The latter and the lack of a net increase in diastolic pressures in vivo are likely due to effects from greater inotropy, which enables hearts to contract to smaller end-systolic volumes and thus have enhanced elastic recoil. Agents with more complex mechanisms of action, such as levosimendan, may also have offsetting effects on diastole owing to PDE3 inhibition and K$_{ATP}$-channel agonism, whereas with more targeted agents (eg, CGP 48506), depressed diastolic function with delayed relaxation may limit their use.

Importantly, because a sensitizer enhances force independently of Ca$^{2+}$ increase, the addition of maneuvers that augment calcium (for example, enhancing heart rate or stimulating sarcoplasmic reticulum (SR) calcium release (PKA activation)) can further augment the inotropic effect in a synergistic manner. This is shown for EMD-57033 in response to a change in heart rate (Figure 4D). Increasing the rate normally stimulates greater contractility by its effects on enhancing SR Ca$^{2+}$ loading and thus release, as well as by rate-dependent effects mediated via TnI phosphorylation. Cotreatment with EMD-57033 amplifies this effect because it targets a more distal mechanism.

The capacity of a sensitizer to exert incremental positive inotropy under conditions of physiological stress was elegantly demonstrated by Tachibana et al. These investigators administered levosimendan to chronically instrumented dogs with tachypacing-induced dilated heart failure. During exercise, untreated animals displayed an increase in preload and little inotropic reserve. Levosimendan enhanced contractility at baseline, but importantly, contractility also further increased during exercise (Figure 5A). As shown in Figure 5B, the inotropic and lusitropic effect of the drug was nearly doubled at peak exercise compared with the benefit observed at rest. This feature of Ca$^{2+}$ sensitizers addresses a major problem with conventional inotropes that work by increasing activator calcium, namely, that the inotropic effect can be better titrated to be less under rest conditions and more prominent when needed during stress.

Figure 5. Impact of levosimendan (LSM) on exercise function in failing canine heart. A. Sample pressure-volume loops show limited systolic reserve (loops shift up to right) and increased preload in congestive heart failure animals without treatment. After receiving levosimendan, the response is altered, so there is now a left shift of the loop with exercise (increased inotropy) and minimal preload recruitment. B. Lusitropic and inotropic response to levosimendan at rest and at varying stages of exercise. There is a synergistic response of the drug during exercise to further enhance beneficial effects on both features of heart function. CHF indicates congestive heart failure. Reproduced from Tachibana et al with permission.

Another important feature of Ca$^{2+}$ sensitizers is their ability to improve contractility without a proportional increase in myocardial energy consumption. Myocardial energy consumption can be divided into 3 categories: ATP used for basal metabolism (protein synthesis, cell maintenance), ATP used for excitation-contraction coupling (primarily SR Ca$^{2+}$-ATPase, Na/K-ATPase), and ATP used for force generation (myosin ATPase). As first demonstrated by Suga, the total consumption is linearly related to total cardiac work (both external and internal work), the latter computed by the pressure-volume area (Figure 6A and 6B). This relation is characterized by a slope (chemomechanical conversion efficiency) and offset (work-independent O$_2$ consumption). Agents that enhance contractility by increasing intracellular
Ca\textsuperscript{2+} increase the offset but do not alter its slope. Drugs that might lower this slope (Figure 6B) would be consistent with a sensitizer-like effect. Alternatively, one can plot the work-independent oxygen cost versus contractility (Figure 6C). In theory, drugs that do not shift the offset yet increase contractility (ie, improving on the energy needs for excitation-contraction coupling) also improve efficiency. This framework has been used to study the calcium sensitizers, as noted below.

Efficiency is often expressed by the ratio of external work to total myocardial oxygen consumption, but such ratios need to be interpreted carefully. As shown in Figure 6D, a pure vasodilator with no inotropic effects can reduce myocardial O\textsubscript{2} consumption (lower PVA) while enhancing external work, resulting in this example in a 46% improvement in efficiency. However, when plotted on a myocardial oxygen consumption–pressure-volume area (MVO\textsubscript{2}–PVA) relation, the data would fall on the same relation and simply reflect the load reduction. The caution is that many Ca\textsuperscript{2+} sensitizers also have potent vasodilator effects due to off-target mechanisms, and this may greatly influence the energetic findings reported in intact hearts with these agents.

Lastly, the magnitude and mechanisms by which efficiency may be improved likely vary depending on the pharmacological mechanism. Drugs that enhance the likelihood of a cross-bridge staying tightly bound (more force per bridge) may improve force without greater m-ATP use or SR-ATP use. However, agents that increase the binding of TnC to calcium could increase cross-bridge formation and raise m-ATP use while still saving on SR-ATP. Antioxidants may improve energetics by affecting the balance of nitric oxide and superoxide\textsuperscript{35} and their respective influences on mitochondrial metabolism.

**Specific Agents: An Update**

Several Ca\textsuperscript{2+} sensitizers have been reported in experimental studies, although only a few have advanced to clinical trials. Levosimendan has advanced the furthest by far in this regard.
and is already approved for intravenous use in several European and South American countries. The only other agent with long-term reported clinical data is pimobendan, approved for use in men but only in Japan. Here, we review data for these and several other of the more prominent agents.

**Levosimendan**

Levosimendan is the most widely studied sensitizer to date and the focus of many recent reviews.\(^5^6\)\(^-\)\(^4^0\) It displays Ca\(^{2+}\)-dependent binding to TnC, with higher affinity at higher calcium concentrations and less effect at low ones, with the primary binding site at the N-terminus of TnC. Here, it appears to stabilize the conformation of the Ca\(^{2+}\)-TnC complex\(^4^1\) to reduce Tnl inhibition of actin/myosin ATPase\(^5^7\) and thereby accelerate the cross-bridge association rate and decelerate the dissociation rate.\(^4^2\) In failing dog hearts, contractility is enhanced by \(\approx 60\%\) based on load-independent pressure-volume analysis.\(^3^3\) In failing human myocardium, increased systolic tension is accompanied by modest dose-dependent increases in the calcium transient, which at high doses is similar to that from milrinone. The importance of calcium-dependent effects, which presumably are not directly related to a sensitizing effect but may be due to PDE3a inhibition, is revealed in acidic conditions, where the inotropic response to levosimendan is blunted largely by loss of Ca\(^{2+}\) transient changes.\(^4^4\) Its capacity to inhibit PDE3 may vary somewhat with species, but data do suggest this is relevant in humans.\(^4^5\) It also activates ATP-sensitive potassium channels, which can have potent effects on coronary tone and contribute to myocardial protection.\(^4^6^,^4^7\) In patients, coronary and systemic vasodilation effects of 45% and 18%, respectively, are observed\(^4^8\) consistent with primary vasodilation mechanisms. Levosimendan can enhance cardiac efficiency, but unloading due to vasodilation may play an important role (ie, Figure 6D). For example, in a canine failure model, the drug increased work-independent cardiac oxygen consumption, and the oxygen cost of contractility was similar for levosimendan as for isoproterenol,\(^4^9\) whereas more favorable effects were reported in isolated guinea pig hearts.\(^5^0\)

In humans, positron emission tomography scanning data found neutral effects on left ventricular efficiency and M\(V\)\(O\)_\(2\).\(^5^1\)

Clinical trials support the acute intravenous efficacy and long-term benefits of levosimendan. In class III-IV heart failure patients, Kivikko et al\(^5^2\) reported that 24-hour administration led to an \(\approx 40\%\) increase in cardiac output, reduced wedge pressure (\(-8.9\) mm Hg), and an \(\approx 30\%\) reduction in systemic vascular resistance, as well as increased heart rate (\(\approx 6\) bpm). These effects were sustained after 48 hours in subjects treated for 24 hours, likely because of the active metabolite OR-1896, which has a half-life of 81 hours,\(^5^3\) 20 times that of levosimendan. This long duration of action may pose limitations to the development of an oral form of the drug. In the LIDO trial (Levosimendan Infusion vs Dobutamine in Severe Low Output Heart Failure),\(^5^4\) 203 patients with severe low-output failure were treated with either levosimendan 0.1 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) or dobutamine 5 to 10 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) for 24 hours. The primary end point of hemodynamic improvement (\(\geq 30\%\) rise in cardiac output and \(\geq 25\%\) fall in pulmonary capillary wedge pressure) was achieved in 28% and 15% of patients receiving levosimendan or dobutamine, respectively (\(P=0.022\)). Importantly, at 180-day follow-up, patients treated with dobutamine had higher mortality (38% versus 26%, \(P=0.029\)). In a comparison with placebo in postinfarction heart failure patients (RUSSLAN, the Randomized Study on Safety and Effectiveness of Levosimendan in Patients With Left Ventricular Failure due to an Acute Myocardial Infarction),\(^5^5\) levosimendan did not affect the primary end point (number of patients developing ischemia or hypotension) but reduced 14-day mortality rates (11.7% versus 19.6% in placebo, \(P=0.03\)) and showed borderline improved mortality at 180-day follow-up (23% versus 31%, \(P=0.053\)).

Preliminary data for 2 additional trials, CASINO (Calcium Sensitizer or Inotrope or None in Low Output Heart Failure Study)\(^5^6\) and REVIVE-1 (Randomized Multicenter Evaluation of Intravenous Levosimendan Efficacy Versus Placebo in the Short-Term Treatment of Decompensated Heart Failure),\(^5^7\) as well as the larger REVIVE-2 and SURVIVE (Survival of Patients With Acute Heart Failure in Need of Intravenous Inotropic Support) trials, have been reported. CASINO studied patients classified as New York Heart Association class IV hospitalized for decompensated failure, with a primary composite end point of death or rehospitalization for worsened failure, and compared levosimendan to dobutamine and placebo at 6 months. The study was terminated after 50% of enrollment (\(n=299\)) because of a survival advantage in the levosimendan treatment group (15.3%, 24.7%, and 39.6% 6-month mortality in the levosimendan, placebo, and dobutamine groups, respectively; \(P=0.04\) for levosimendan versus placebo). REVIVE-1 studied 100 patients and used a composite end point of patient status (improved: Self-reporting; worsened: Death or required intravenous treatment for heart failure during the 5-day trial duration; or unchanged: Neither improved nor worsened).\(^5^7\) A borderline benefit of levosimendan (12 \(\mu\)g/kg bolus, followed by 24-hour 0.2 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) over placebo was reported (\(P=0.043\)). The recently presented REVIVE-2\(^5^8\) (\(n=600\)) also used the same design. By day five, 33% more patients in the levosimendan group improved, and 30% fewer worsened than in the placebo control group (\(P=0.015\)). However, 90-day all-cause mortality was not improved (15.1% versus 11.6%), and there were safety concerns, with a higher incidence of arrhythmia and hypotension in subjects receiving levosimendan. The SURVIVE trial evaluated 1327 patients with acute decompensated heart failure and mean EF <30%. All were judged to require dobutamine after failing to respond to vasodilators or diuretics, so this was a particularly sick group. Patients were randomized to dobutamine or levosimendan, with the primary end point being all-cause mortality after first infusion at 180 days. There was no significant difference in mortality (26% levosimendan versus 28% dobutamine) or at 31 days (secondary end point). Complications (hypotension, tachycardia, adverse renal events) were also similar between treatments. Taken together, the data suggest symptomatic improvement with levosimendan in the short term in acutely decompensated subjects but no impact on mortality over placebo, with similar mortality rates when compared to dobutamine.
Pimobendan
Pimobendan is a combination calcium sensitizer and PDE3 inhibitor.60,61 It was tested in heart failure patients in the late 1990s,62 but remains clinically approved in Japan only. Pimobendan lowers the Ca\(^{2+}\) level required for actin sliding in an in vitro motility assay using reconstituted thin filaments.63 Clinical data showed benefits on exercise capacity but also a trend toward increased mortality,62 and further clinical development has not been reported.

EMD-57033
EMD-57033 has potent Ca\(^{2+}\)-sensitization effects but is also a PDE3 inhibitor, the latter appearing at higher but pharmacologically relevant doses. Clinical development was ultimately halted because of bioavailability limitations, but experimental studies provided many useful insights into the general drug class as noted in Figures 2 and 5. A recent further finding revealed greater potency of EMD-57033 in a mouse model in which a truncated TnI was overexpressed to mimic proteolytic changes reported in myocardial stunning.64,65 which suggests that such drugs may be particularly suited to diseases with abnormal Ca\(^{2+}\) sensitivity from altered regulatory thin-filament proteins. This would likely depend on the precise mechanism of action of the agent.

MCI-154
MCI-154 increases actin sliding velocity in in vitro motility assays using reconstituted thin filaments and is thought to lower the threshold Ca\(^{2+}\) for Tm-Tn complex activation.65 The agent also acts as a PDE3 inhibitor, which likely contributes to vasodilator and other effects observed in vivo.66 In isolated canine hearts, MCI-154 increased non–work-dependent oxygen consumption less than with matched inotropic stimulation by PDE3 inhibition or Ca\(^{2+}\) stimulation.67 Similar data were reported in humans with mild heart failure in a comparison with dobutamine.68 However, recent unpublished data have failed to support its clinical use, and further development has been suspended.

Miscellaneous
Several other agents designed to enhance myofilament response independent of trigger calcium should be noted. One is Org-30029, which has been shown to increase calcium sensitivity in canine ventricular trabeculae,69 but like EMD-57033, to do so following a pattern depicted in Figure 1A, ie, with increases in both systolic and diastolic force in both nonfailing and failing human myocardium.70 Its clinical use remains to be clarified but has been troubled by dose-dependent side effects (nausea and vomiting). Another agent is DPI 201-106, which enhances myofilament myosin ATPase-Ca\(^{2+}\) sensitivity more in failing than in normal myocardium.70 However, this agent also is a sodium channel activator that induces a CAP-dependent inotropic effect that can result in significant cardiac arrhythmia,71 and it has not been clinically useful.

More recently, a new approach has been taken to screen molecules that directly activate the cardiac myosin ATPase,72,73 Compounds such as CK-1213296 and CK-1827452 have been reported to accelerate the transition of myosin into the force-generating state and enhance contractility without altering intracellular Ca\(^{2+}\). These drugs have positive inotropic effects in a variety of heart failure models, including infarcted rats and tachypacing- and pacing-plus-infarction–induced heart failure in dogs. They are not associated with systemic vasodilation, and they can improve diastolic function in vivo. Rather than being termed Ca\(^{2+}\) sensitizers, such drugs are considered myosin activators because the target is the myosin molecule itself, devoid of any of the thin-filament, Ca\(^{2+}\)-regulated sarcomeric proteins.

Antioxidants (Oxypurinol)
Oxidant stress is thought to play an important role in myocardial dysfunction. Its pathophysiological role was first elucidated in ischemia/reperfusion studies, in which antioxidants improved myocardial stunning, and this role has since been expanded to most forms of heart failure. Oxygen radicals may depress myocardial function by their direct actions on Ca\(^{2+}\) sensitivity, perhaps involving oxidation of myofibrillar proteins.74 Xanthine oxidase inhibitors such as allopurinol induce a modest left shift of the force-Ca\(^{2+}\) relation with an increase in maximal activated force in stunned isolated muscle fibers.75 This is accompanied by reduced Ca\(^{2+}\) transient, which suggests a mechanism for improving myocardial efficiency. The latter was observed in intact failing canine hearts76 and was shown to depend on a balance between oxidative stress and nitric oxide synthase activity.77 This effect is not specific to the antioxidant, however, as similar findings were observed with ascorbic acid.78 Human studies supported modest energetic benefits from allopurinol but did not find changes in pump function or contractility.

Investigators recently completed a randomized trial of oxypurinol (the active allopurinol metabolite) for the treatment of congestive heart failure.79 The trial (OPT-CHF, or Oxypurinol Therapy for CHF) was a double-blind, placebo-controlled trial of 405 New York Heart Association class III-IV heart failure patients. However, the study data released by the trial’s sponsor (Cardiome, Vancouver, Canada) in August 2005 revealed no significant benefits (P=0.357) on the primary composite end point (functional class, patient status, and clinical events indicating worsened heart failure). Uric acid levels did fall in the patients receiving oxypurinol. Thus, a role for antioxidant approaches remains unclear.

Conclusions
The search for novel approaches to improve cardiac function in heart failure is presently receiving a long-needed boost with the development of agents that can enhance contraction without requiring greater activator Ca\(^{2+}\). Although few existing agents have made it to successful clinical trials, levosimendan maybe poised for this. Others, such as the myosin enhancers, are just entering clinical investigation. Whether pure myofilament activation mechanisms are ideal, or whether having some off-target effects such as K\(_{ATP}\) channel agonism or even some PDE3 inhibition is ultimately optimal, remains to be clarified. Nonetheless, this class of drugs offers promise and hopefully will provide a useful therapeutic
approach for both acute and chronic management of heart failure in the relatively near future.

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Disclosures

Drs Kass and Solaro are both members of the scientific advisory board of Cytokinetics. Dr Kass has received research funding from Cytokinetics. Cytokinetics is a manufacturer of novel sarcomere function enhancing agents that are currently entering clinical trials.

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