Calcium-Sensitizing Agents in the Failing Heart

An ESSAY

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ABSTRACT

For acute worsening of heart failure, the short-term administration of Levosimendan, a new inotrope with calcium sensitizing properties, appears to be safer than Dobutamine. Also, in acute heart failure after myocardial infarction, Levosimendan improved symptoms and halved mortality during the first 72h, a difference in mortality which was maintained over the next6 months. For the first time, a positive inotropic drug seems to have beneficial effects on long-term survival in patients with acute decompensated heart failure.

Key words:Heart failure-Inotropes-Calcium sensitizers-Levosimendan

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LIST OF ABBREVIATIONS

AC	Adenyl Cyclase
ACE	Angiotensin Converting Enzyme
ADH	Antiduretic hormone
BNP	B-type Natriuretic Peptide
cAMP	Cyclic Adenosine Monophosphate
CHF	Congestive Heart Failure
cTn	Troponin complex
DAG	Diacylglycerol
EGF	Epidermal Growth Factor
IP3	Inositol triphosphate

IV	Intravenous
LVSD	Left Ventricular Systolic Dysfunction
MHC	Myosin Heavy Chain
MI	Myocardial Infarction
MLC	Myosin Light Chain
MyBp-C	Myosin-binding protein C
NSAIDs	Non Steroidal Anti Inflammatory Drugs
NYHA	New York Heart Association
PAP	Pulmonary Artery Pressure
PCWP	Pulmonary Capillary Wedge Pressure
PDE3a	Phosphodiesterase 3A
PKA	Protein Kinase A
PLC	Phospholipase c
PVR	Pulmonary Vascular Resistance
SR	Sarcoplasmic Reticulum
TGF-beta	Transforming Growth Factor beta
Tm	Tropomyosin

Excitation/Contraction Coupling

Physiological and pharmacological modifications in myofilament response to Ca^{2+} occur as an integral mechanism of excitation/contraction coupling.

In diastole, Ca^{2+} concentrations surrounding the sarcomere of working heart muscle cells fall below the myofilament activation threshold. With electrical stimulation, Ca^{2+} move into the cytoplasmic space and binds to the myofilaments, inducing force and contraction that are about 20% to 25% of maximum. One way to increase contractile force is to increase the Ca^{2+}that enters the myofilament space, and this is physiologically achieved by an elaborate set of membrane-related mechanisms that control Ca^{2+}fluxes. (9)

Alternatively, one can regulate how the sarcomere responds to Ca^{2+}. Normally, changes in sarcomere length and the phosphorylation of regulatory proteins associated with thin and thick filaments modify Ca^{2+} sensitivity and rates of filament sliding, thereby modifying contractile reserve in concert with Ca^{2+}flux to and from the sarcomeres. Pharmacological approaches that further enhance the sarcomeric Ca^{2+} response modify this normal control mechanism. (10)

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 1 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.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 troponin complex (cTn) to the actin-Tm thin-filament backbone. (11)

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+}/Mg binding sites at its

C-terminal lobe (*Figure 2*). 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.

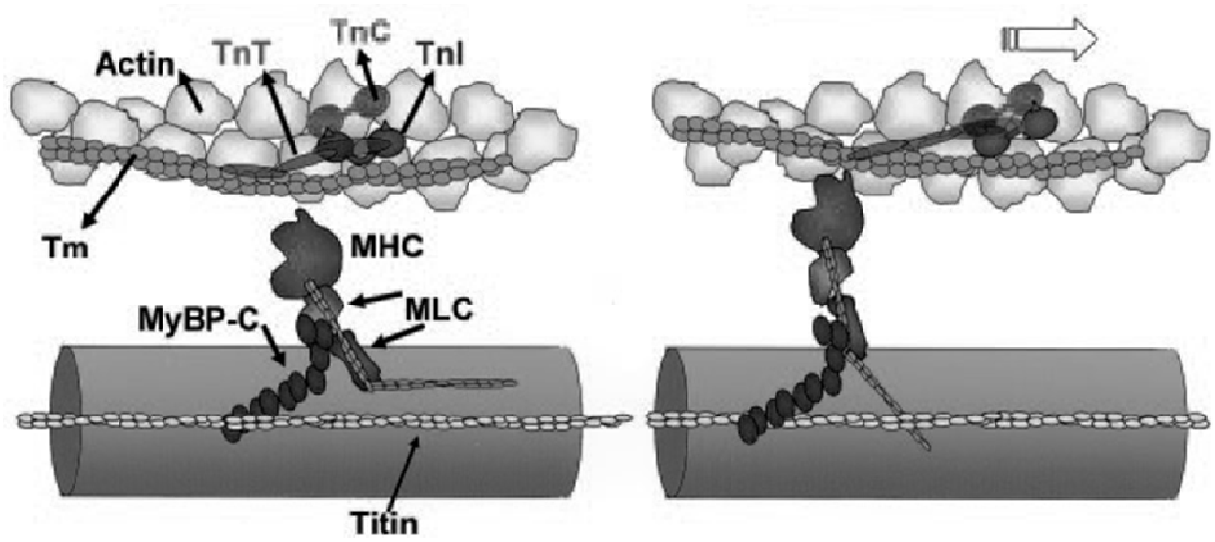

Figure 1*. A structural unit of the sarcomere with thick-filament cross-bridges (myosin heavy chain, MHC) shown in diastole (left) and reacting with thin-filament actin in systole (right). In diastole, Tm (tropomyosin) blocks the crossbridge formation. In the absence of Ca^{2+} bound to the N-lobe of TnC, Tm is held in this position by protein-protein interactions that involve TnI, 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 TnI, 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 titin, a molecular spring protein in the sarcomere.*[(11)]

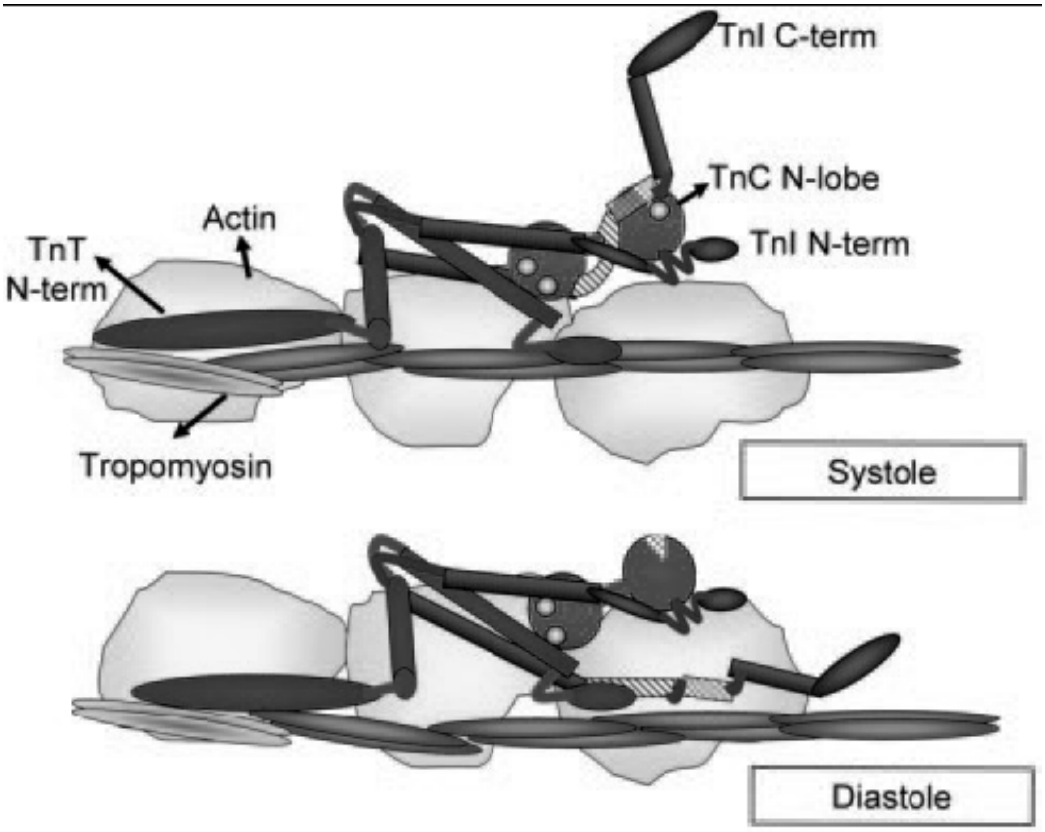

***Figure 2**. Molecular mechanism of thin-filament activation. In diastole, Tm is held in blocking position by its interactions with TnT and by interactions of a TnI inhibitory peptide and switch peptide with actin. Ca^{2+} binding to the N-lobe of cTnI induces the opening of a hydrophobic"sticky" patch on TnC that reacts with the switch peptide of cTnI, releasing Tm and the thin filament from the inhibited state. Cardiac TnI contains a unique N-terminal peptide that also reacts with the N-lobe of TnC and regulates Ca^{2+} binding affinity. Term indicates terminal.*[(11)]

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 2*), 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, troponin is tethered to actin-Tm by the binding of 2 regions of cTnI with actin (inhibitory peptide and a second actin-binding region) and by an interaction of the N-terminal tail of cTnT to actin-Tm. The 2 actin-binding regions of cTnI flank a small hydrophobic helix (the switch peptide) that binds to the hydrophobic sticky patch formed in the cTnC N-lobe on Ca^{2+} binding to the regulatory site. These protein-protein interactions affect the structure of actin and restrict the range of movements of Tn-Tm on the thin filament, impeding the actin– cross-bridge reaction.(11)

Ca^{2+} binding to cTnC triggers systole by inducing movements of the thin-filament proteins so that blocked crossbridges may react with actin in a cycle of interactions that impel the thin filaments on each side of the sarcomere toward the center (*Figure 1*). A key aspect of the molecular mechanism that triggers contraction is a Ca^{2+}-induced hydrophobic left or sticky patch on the N-lobe of cTnC.(12)

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 actinbinding 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 3A), which indicates an underlying cooperative process. 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 later on to how this interaction can be modified by targeted pharmacological interventions. (13)

The Myofilament Force-Ca^{2+} Relationship

A common approach to assessing myofilament Ca^{2+} activation is to apply varying Ca^{2+} concentrations (about 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 Ca^{2+} level.

Alternatively, intact trabeculae can be studied by chemically inactivating intracellular Ca^{2+} fluxes, opening sarcolemmal Ca^{2+} channels, and tetanically stimulating the muscle while varying extracellular Ca^{2+}concentration.(14)