The Cardiac Myosin Activator, CK-1122534, Overcomes the Myosin II Inhibitors 2, 3-Butanedione-2-Monoxime and (-)-S-Blebbistatin without Affecting the Ca^{2+} Transient in Ventricular Myocytes

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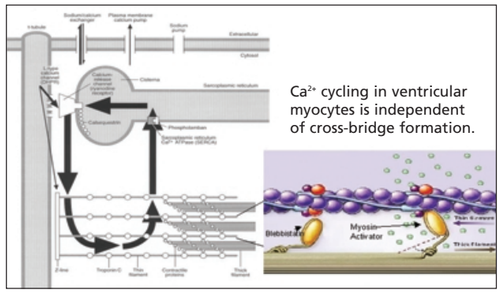
Abstract

We have previously demonstrated that myosin activators increase cardiac myocyte contractility without altering the calcium transient and do so independently of second messenger activation. To further characterize this novel mechanism, we examined the myosin activator, CK-1122534 with identified myosin inhibitors. The well-known myosin inhibitor 2, 3-butanedione-2-monoxime (BDM) has been extensively used in myocyte biology, while the recently identified myosin inhibitor (-)-S-blebbistatin effects on cardiac myocytes are unknown. Both inhibitors uncouple the myosin-actin cross-bridge contractility from the Ca^{2+} transient so that at high concentrations, no contractility is observed but the calcium transient continues to cycle. These data show that myosin inhibitors, BDM and blebbistatin, act similarly to decrease myocyte contractility by uncoupling the contractility and Ca^{2+} transient mechanisms and, in this physiological system, blebbistatin is 1000 times more potent than BDM. Inhibition of contractility by these compounds is overcome by the myosin activator CK-1122534 with no alteration of the Ca^{2+} transient. These results are consistent with the mechanism of action of myosin activators and support that cardiac myosin activators may be useful therapeutics for treating heart failure.



Introduction

The cellular structures involved in excitation-coupling (EC coupling) are shown above. Excitation causes a small amount of calcium to enter the cell at the voltage-dependent L-type calcium channel. This small increase in the calcium concentration induces a larger amount of calcium release, calcium induced calcium release (CICR), from the sarcoplasmic reticulum (SR) via the ryanodine receptor. This calcium now acts at the level of the sarcomere to induce contraction. At the end of the contraction, calcium is pumped back into the SR via sarco/endoplasmic reticulum calcium ATPase (SERCA).

Traditional inotropic agents used in treating acute decompensated heart failure increase cellular contractility by increasing the calcium transient but these therapies result in increased mortality in clinical trials (1, 2).

We have identified compounds, with a novel mechanism of action, that directly activate cardiac myosin itself (3, 4). Myosin activators specifically modulate cardiac myosin ATPase without affecting the activity of skeletal, smooth, or non-muscle myosin as well as not altering the Ca2+ transient.

BDM is a well known myosin inhibitor that decreases contractility in the cardiac myocyte by uncoupling the calcium transient and contraction process (5) while a more recent myosin inhibitor blebbistatin (6, 7) has not been examined in cardiac systems.

Methods

Cells:
Myocytes were isolated from adult male Sprague Dawley rats using a collagenase digestion procedure and made Ca^{2+} tolerant to 1.5mM in Tyrode buffer. Myocytes were perfused in Warner chambers warmed to 37°C, field stimulated at 1Hz and 10V.

Analysis:
Contractility average transients were analyzed using the Ionwizard analysis program to determine changes from end diastolic length, fractional shortening (% decrease from the end diastolic length) and maximum contraction and relaxation velocities (µm/sec). The averaged calcium ratio transients were analyzed using the Ionwizard analysis program to determine changes in diastolic and systolic ratios and the 75% time to baseline (T75).

Ca2+ Transient:
Myocytes were loaded with 1uM fura-2, 500mM probenecid in Tyrode buffer and simultaneous contractility and fura-2 ratios determined using an IonOptix system modified for fluorescence analysis.

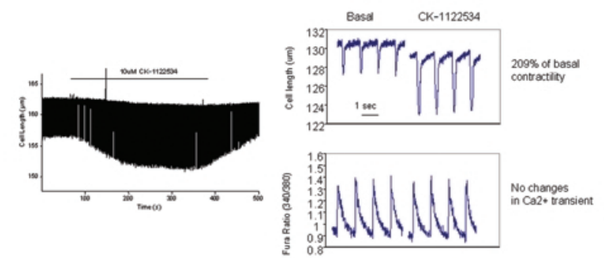
Reagents:
CK-1122534 was synthesized by Cytokinetics Inc., blebbistatin (Toronto research), fura -2 AM ester and pluronic (Molecular Probes), probenecid and salts obtained from Sigma.

Objectives

- To determine the effect of the myosin inhibitor, blebbistatin, in a physiological system (contracting myocyte)
- Characterize the MOA of a cardiac myosin activator CK-1122534 with co-treatment with myosin inhibitors

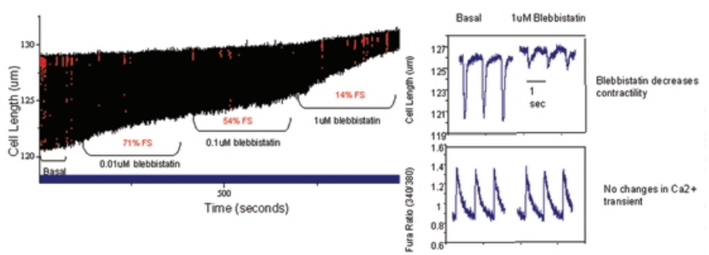
Results

1. Myosin activators, such as CK-1122534, increase contractility and do not alter the calcium transient

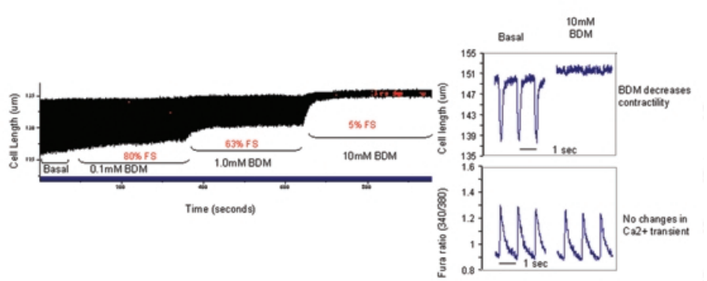


Individual tracings showing increased contractility and no change in the calcium transients after CK-1122534 treatment

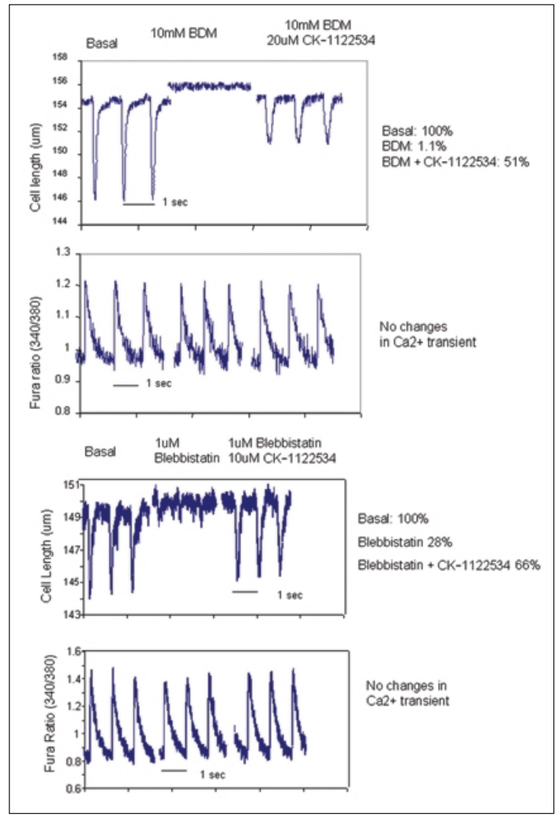
2. Myosin inhibitors, BDM and blebbistatin, decrease basal contractility in contracting myocytes and does not change the Ca^{2+} transient



Individual tracing showing a dose responsive inhibition of myocyte contractility but with no change to the calcium transient with blebbistatin (above) and BDM (below).



3. CK-1122534 overcomes the inhibition of myosin inhibitors blebbistatin and BDM



Individual tracings showing that BDM and blebbistatin treatment decreases contractility by uncoupling the calcium transient. Administration of the myosin activator CK-1122534 partially restores myocyte contractility.

Acknowledgements

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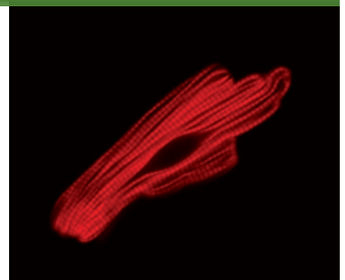

Summary/Conclusions

- Blebbistatin and BDM inhibit ventricular myocyte contractility in a dose dependent manner
- Blebbistatin is ~1000X more potent than BDM in this physiological system
- The myosin activator, CK-1122534, overcomes inhibition by blebbistatin and BDM

These results are consistent with the on-target mechanism of action of cardiac myosin activators, such as CK-1122534

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