Effects of Cardiac Myosin Activators on Excitation-Contraction (E-C) Coupling in Ventricular Myocytes

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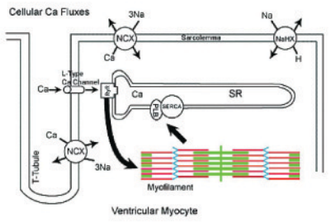
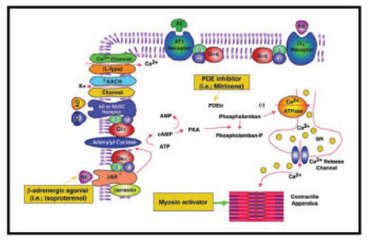
Introduction





Excitation-Contraction (E-C) coupling (above left) refers to the events that transfer an action potential (membrane electrical event) into a mechanical event (contraction). In cardiac muscle, electrical excitation of the surface membrane leads to an action potential that is propagated as a wave of depolarization along the surface and along the transverse tubules. The depolarization of the L-type calcium channels result in a small amount of calcium entering the cell and this induces the release of large amounts of calcium from the sarcoplasmic reticulum (SR) via the ryanodine receptors (RyR). The calcium released from the SR then binds troponin C which, in turn, allows binding of the myosin heads to actin and subsequent contraction. The large amount of calcium released by the SR is then pumped back into the SR via the SR calcium ATPase pump (SERCA) or extruded from the cell by the sodium calcium exchanger (NCX). Cellular calcium movement is further complicated by the presence of ion channels and transport systems that may play a role in mediating calcium release (ref 1).

Myosin activators are small molecule agents that directly stimulate the activity of the cardiac myosin ATPase in the sarcomere. In contrast to current inotropic agents for the treatment of congestive heart failure (above right), myosin activators increase contractility without altering the calcium transient (ref 2-5). This is an important aspect of the myosin activator mechanism of action (MOA) because increasing intracellular calcium increases ATP use to maintain ionic balance and can cause electrical instability, which respectively can cause ischemia and arrhythmias, and poor clinical outcomes (ref 6, 7).

The objective of this study was to examine aspects of E-C coupling in adult rat cardiac myocytes after treatment with the myosin activators CK-1827452 (currently in clinical trials for the treatment of heart failure) and CK-1316719. This included determining the calcium transient and contractility parameters at 0.5 Hz, and effects of CK-1316719 on sarcoplasmic reticulum calcium content and on the sodium calcium exchanger.

Methods

Myocyte contractility experiments: Left ventricular myocytes were isolated from adult male SD rats (275-325g) using a collagenase digestion procedure and used within 5 hrs of isolation. Myocytes were warmed in perfusion chambers, perfused with Tyrodes buffer and field stimulated at 0.5 Hz. To determine contractility, myocytes were imaged through a 40x objective and the images were digitized at a sampling speed of 240 Hz. Frame grabber, myopacer, acquisition, and analysis software were obtained from IonOptix (Milton, MA). After an initial 5 min basal contractility period, compounds were perfused for 5 minutes. Fractional shortening was monitored continuously.

Calcium transient analysis: Myocytes were loaded with 2 µM fura-2 AM (Molecular Probes) and simultaneous contractility and fura-2 ratios determined using an IonOptix system modified for fluorescence analysis.

Cell Analysis: For each cell, ten or more contractility and calcium ratio transients at basal and 5 min after compound addition, were averaged and compared. Contractility transients were analyzed using the IonWizard analysis program to determine changes in diastolic length, maximum contraction and relaxation velocities, fractional shortening (% change in the diastolic length; FS) and time to peak. Data were normalized to basal values (basal equals 100%) and expressed as % of basal.

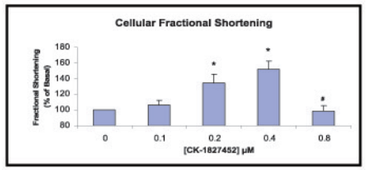
SR content: Fura-2 loaded myocytes were perfused with Tyrodes buffer containing CK-1316719 or 20nM isoproterenol and paced at 0.5 Hz at 22°C. After 5 min exposure to compounds, pacing was stopped and 10mM caffeine in Tyrodes buffer was applied to the myocyte using a SF-77B perfusion fast step system (Warner Instruments) while intracellular calcium was continuously monitored.

NCX protocol: Experiment was performed as per the SR content protocol except that Tyrodes buffer was modified to lack calcium and substituting lithium for sodium.

Statistics: Data are mean +/- SEM. Statistics were performed using the Students *t*–test and P<0.05 was considered significant.

Reagents: CK-1316719 and CK-1827452 provided by the Cytokinetics Chemistry department. All other reagents not detailed above were from Sigma.

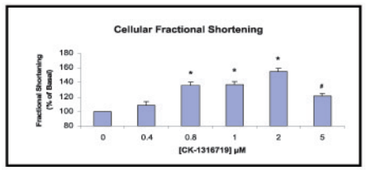
Myosin activators increases cellular contractility…



CK452 µM	Cell Length (% of basal)	Fractional Shortening (% of basal)	Time to Peak (% of basal)	Contraction Velocity (% of basal)	Relaxation Velocity (% of basal)
0	100	100	100	100	100
0.1	99.5 +/- 0.1	106.7 +/- 5.8	114.2 +/- 1.2*	92.1 +/- 5.2	103.5 +/- 9.1
0.2	97.8 +/- 0.6	134.6 +/- 11.5*	156.1 +/- 7.4*	88.9 +/- 8.4	100.5 +/- 10.7
0.4	95.9 +/- 0.7	152.2 +/- 10.5*	187.8 +/- 9.1*	87.5 +/- 8.7	124.5 +/- 11.9
0.8	89.6 +/- 0.9#	98.7 +/- 6.7	243.7 +/- 9.3*	37.3 +/- 4.1*	49.3 +/- 5.4*

* p < 0.05 compared to basal

Figure 1. Treatment with CK-1827452 (CK452, above) or CK-1316719 (CK719, below) increases fractional shortening and the time to peak parameter (green box) in a dose responsive manner in adult cardiac myocytes. The increase in time to peak contraction is an indication of increased duration of contraction, a characteristic of the myosin activator MOA. At the highest dose, fractional shortening is attenuated due to shortening of diastolic cell length (# = > 5% decrease from basal). N = 10



CK719 µM	Cell Length (% of basal)	Fractional Shortening (% of basal)	Time to Peak (% of basal)	Contraction Velocity (% of basal)	Relaxation Velocity (% of basal)
0	100	100	100	100	100
0.4	99.7 +/- 0.1	108.8 +/- 4.7	111.1 +/- 2.2	100.9 +/- 3.7	111.9 +/- 6.2
0.8	98.7 +/- 0.2	136.4 +/- 3.8*	130.5 +/- 3.2*	105.9 +/- 3.8	138.2 +/- 5.9*
1	98.4 +/- 0.2	137.2 +/- 4.4*	133.9 +/- 3.0*	103.8 +/- 3.8	131.4 +/- 5.3*
2	96.3 +/- 0.4	154.6 +/- 4.8*	155.6 +/- 2.9*	100.5 +/- 4.2	143.1 +/- 3.8*
5	89.2 +/- 0.4#	121.3 +/- 3.7*	241.2 +/- 5.6*	44.4 +/- 2.3*	84.9 +/- 5.8

* p < 0.05 compared to basal

…without increasing the calcium transient

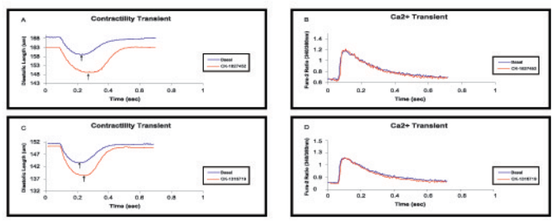
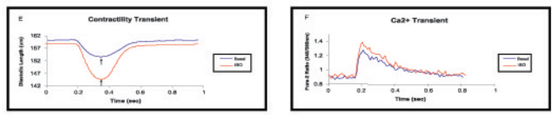


Figure 2. Representative tracings demonstrating that the myosin activators CK-1827452 (400 nM) and CK-1316719 (1 µM) increase cardiac myocyte contractility (A and C) without increasing the calcium transient (B and D). Arrows denote time of peak contraction. Note that myosin activators increase the time spent in contraction compared to basal. In contrast below, the β-adrenergic agonist isoproterenol (ISO, 2 nM) increases contractility by increasing the Ca2+ transient (E and F, respectively).



Myosin activators do not change SR calcium content or alter the sodium calcium exchanger

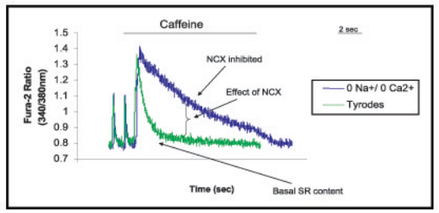


Figure 3. SR calcium content and sodium calcium exchanger (NCX) procedure. To measure SR calcium content, pacing is stopped and caffeine (10mM) administered to open the ryanodine receptors and release the entire contents of the SR into the cell. To determine the contribution of the sodium calcium exchanger (NCX), both sodium and calcium are removed from the caffeine pulse buffer so the sodium calcium exchanger is inhibited in both directions. In this blocked system, the difference between the released SR content and total observed calcium is due to the activity of the sodium calcium exchanger.

In the following figures, cardiac myocytes were challenged for 5 minutes with the β-agonist isoproterenol (Figure 4) or CK-1316719 (Figure 5) and the SR content determined. The effect of CK-1316719 treatment on the sodium calcium exchanger is shown in figure 6. All cells were paced at 0.5 Hz prior to caffeine administration.

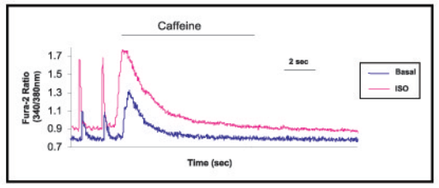


Figure 4. Isoproterenol treatment (20 nM) results in both increased calcium transients and SR calcium content due to phosphorylation effects on phospholamban and the L-type calcium channel.

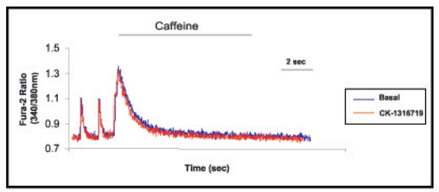


Figure 5. In contrast, treatment with CK-1316719 (1 µM) does not alter the SR calcium content.

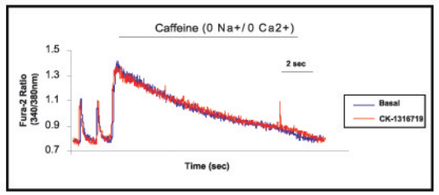
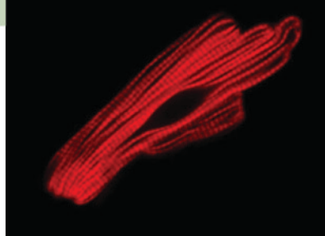


Figure 6. The NCX is unchanged after CK-1316719 (1 µM) treatment.



Conclusions

1) The cardiac myosin activators CK-1827452 and CK-1316719 increases myocyte contractility by increasing the duration of the contraction.
2) Cardiac myosin activators do not increase intracellular calcium.
3) Treatment with CK-1316719 does not change sarcoplasmic reticulum calcium content or alter the sodium calcium exchanger.

These data support the proposed mechanism of action of myosin activators—increasing contractility by directly activating cardiac myosin—with no effects on other aspects of E-C coupling including calcium dynamics.

References

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