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Effects of the Cardiac Myosin Activator CK-1316719 on Excitation-Contraction (E-C) Coupling in Ventricular Myocytes

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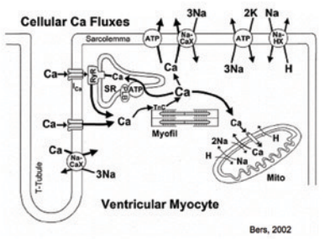
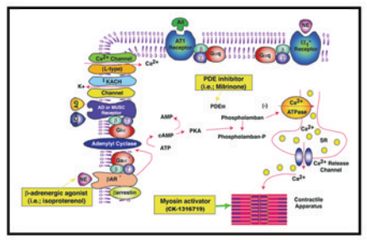
Introduction





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 (NaCaX). 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 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) since inotropic agents that do increase contractility by increasing calcium have 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 activator CK-1316719.This included determining the calcium transient and contractility parameters at 0.5 Hz, and effects of CK-1316719 on sarcoplasmic reticulum 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 uM 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 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. The averaged calcium ratio transients were analyzed to determine changes in fura-2 diastolic and systolic ratios and the 75% time to baseline (T_{75}).

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.

NaCaX 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 provided by the Cytokinetics Chemistry department. All other reagents not detailed above were from Sigma.

CK-1316719 increases cellular contractility…

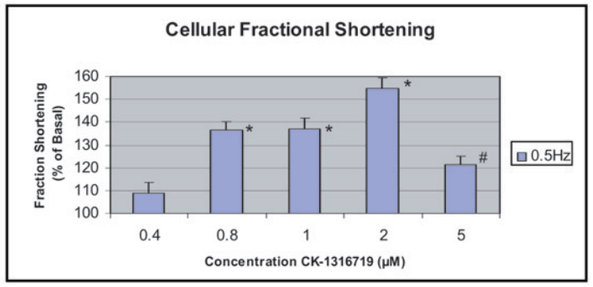


Figure 1. Treatment with CK-1316719 increases fractional shortening in a dose responsive manner in adult cardiac myocytes paced at 0.5 Hz. At the highest dose fractional shortening is attenuated due to shortening of diastolic cell length (# = > 5% decrease from basal) detailed below (n = 12).

0.5Hz 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

Table 1. Group data demonstrating dose dependent increases in fractional shortening after CK-1316719 treatment correlating with the time to peak parameter in myocytes paced at 0.5 Hz. The increase in time to peak contraction (green box) is an indication of increased duration of contraction, a characteristic of the myosin activator MOA. No significant change in contraction velocities were observed except at the highest doses. Relaxation velocities were modestly increased.

…without increasing the calcium transient

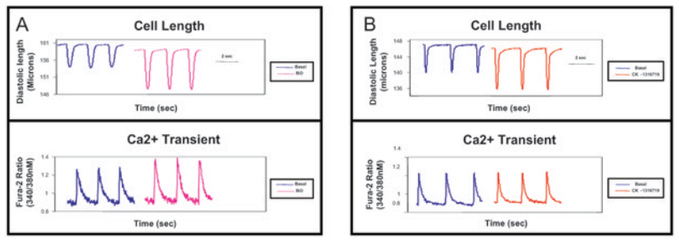


Figure 2. Representative tracings of contractility transient and corresponding calcium transient (fura-2 ratios) before and after treatment with 2 nM isoproterenol (ISO; A) or 1 μM CK-1316719 (B) in myocytes paced at 0.5 Hz. In both groups, increased contractility is observed. Isoproterenol treatment increased the calcium transient. In contrast, CK-1316719 had no effect on the calcium transient. Group data are shown below.

	n	Fractional Shortening (% of basal)	Diastolic Ratio (340/380nm)	Systolic Ratio (340/380nm)	Time to Baseline 75% (sec)
Basal		100%	0.84 +/- 0.01	1.27 +/- 0.02	0.32 +/- 0.01
1uM CK-1316719	6	165.3 +/- 8.3*	0.79 +/- 0.02	1.21 +/- 0.01*	0.33 +/- 0.02
Basal		100%	0.95 +/- 0.02	1.24 +/- 0.01	0.26 +/- 0.02
2nM ISO	4	156.5 +/- 9.1*	0.98 +/- 0.03	1.41 +/- 0.04*	0.22 +/- 0.01

CK-1316719 does not change SR calcium content or alter the sodium calcium exchanger

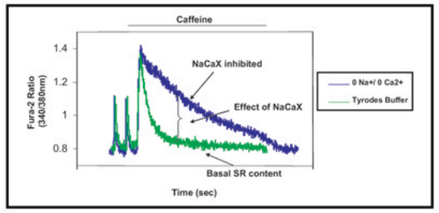


Figure 3. SR calcium content and sodium calcium exchanger (NaCaX) procedure. To measure SR calcium content, pacing is stopped and caffeine (10mM) administered to open the ryanodine receptors and release the entire contents of the SR into the cell. To determine the contribution of the sodium calcium exchanger (NaCaX), 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.

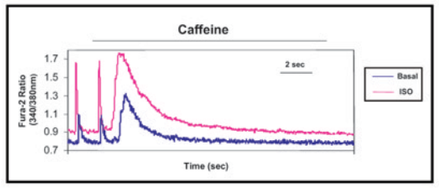


Figure 4. Isoproterenol treatment (20 nM) results in both increased calcium transients and SR content due to phosphorylation effects on phospholamban and the L-type calcium channel.

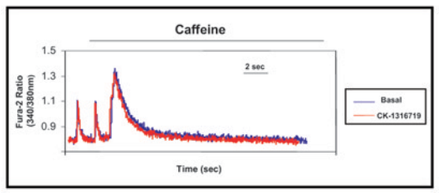


Figure 5. In contrast, treatment with CK-1316719 (1 μM) does not alter the SR content.

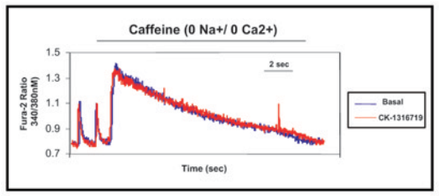
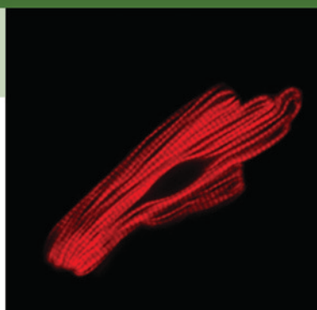


Figure 6. The NaCaX is unchanged after CK-1316719 (1 μM) treatment.



Conclusions

1) The cardiac myosin activator CK-1316719 increases contractility (0.5 Hz) in a dose-responsive manner – without increasing intracellular calcium
2) Treatment with CK-1316719 does not change sarcoplasmic reticulum calcium content
3) Treatment with CK-1316719 does not 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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