

CELLULAR RESPONSES OF THE MYOSIN ACTIVATOR CK-0689705 IN NORMAL AND HEART FAILURE MODELS

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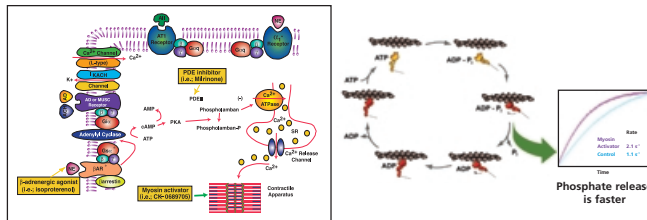
Abstract #1501

ABSTRACT

A hallmark of heart failure (HF) is decreased cardiac contractility. Previous efforts to increase contractility have included agents with direct (β -adrenergic agonists; isoproterenol, ISO) or indirect (phosphodiesterase inhibition, PDE) effects on the calcium transient. A novel approach to improving cardiac contractility is to directly activate cardiac myosin. We now report on CK-0689705, a small molecule that activates the cardiac myosin ATPase, but not the skeletal or smooth muscle myosin ATPases in reconstituted soluble biochemical assays. The cellular response to CK-0689705 was determined in adult rat ventricular myocytes isolated from Sprague-Dawley rats and rats with defined HF. Cellular contractility was quantified using edge detection and the calcium transient measured using fura-2 loaded myocytes. CK-0689705 increased contractility in a dose dependent manner with no significant increases in the calcium transient. In contrast, ISO significantly increased the calcium transient and contractility. Contractility induced with CK-0689705 was additive to increases induced by ISO with no further increase in the calcium transient, demonstrating CK-0689705 is not a PDE inhibitor. HF animals (myocardial infarction was induced by left coronary ligation) had decreased contractility *in vivo* and increased heart size (hypertrophy). Myocytes from HF rats had significantly truncated responses to isoproterenol, compared to cells from sham animals, due to β -adrenergic down regulation, a characteristic of HF at the cellular level. In the same cells, there was a significant increase in contractility to CK-0689705 equivalent to the response observed in sham cells. Results demonstrate that the biochemically defined myosin activator CK-0689705 increases contractility without affecting the calcium transient. Additionally, CK-0689705 increases contractility equally well in cells isolated from animals with identified HF. These results suggest that cardiac myosin activators, such as CK-0689705, may be useful therapeutics for treating heart failure.

INTRODUCTION

Traditional inotropic agents used in treating acute decompensated heart failure increase cellular contractility by increasing the calcium transient. β -adrenergic agonists activate β -adrenergic receptors resulting in an increase in cAMP and activation of the PKA signaling cascade. Numerous proteins are phosphorylated including phospholamban that results in an increase of the calcium transient and thus contractility. Phosphodiesterase (PDE) inhibitors increase the cAMP concentrations by slowing cAMP degradation also resulting in a calcium transient increase. PDE inhibiting compounds result in increased mortality in clinical trials likely due to altering the calcium transient (Packer *et al*, 1991; Cohn *et al*, 1998).



Myosin activators, via a distinct and novel mechanism, directly stimulate activity of the myosin ATPase in the cardiac sarcomere. *In vitro* enzymatic assays demonstrate that myosin activators accelerate the rate limiting step of the myosin enzymatic cycle, reported by phosphate release, almost 2- fold. This portion of the cycle constitutes transition from the weakly to the strongly bound state of myosin. Thus, by reducing the time spent in the weakly bound state, myosin activators shift the myosin enzymatic cycle in favor of the strongly bound, force producing state. See abstract 147 for biochemical details.

OBJECTIVE

Examine a myosin activator, CK-0689705 for activity in cells from normal and heart failure animals.

MATERIALS & METHODS

Animals: Adult male Sprague Dawley rats were used in all experiments. For characterization of CK-0689705, cells from 275-325 g normal rats were utilized. For HF experiments, rats had the chest opened and the left coronary artery ligated, resulting in an anterior wall myocardial infarction (MI). For sham treatment, rats had the chest opened, artery exposed but not ligated. Sham and HF animals were used 8 weeks post-surgery.

Echocardiography:

The cardiac function (fractional shortening) of MI and sham anesthetized rats (isoflurane) was determined by echocardiography with a GE System V and 10 MHz probe.

Myocyte contractility experiments:

Ventricular myocytes were isolated from rats using a collagenase digestion procedure and used within 5 hrs of isolation. Myocytes were warmed in perfusion chambers, perfused with Tyrode buffer and field stimulated at 1 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 1 min basal contractility period, compounds were perfused for 5 minutes before a 2 min washout period. In HF experiments, cells were perfused for 5 min with 5 μ M CK-0689705, a washout period, and then with 20 nM isoproterenol.

Calcium transient analysis:

In some cases, myocytes were loaded with 1 μ M fura-2, AM (Molecular Probes) and simultaneous contractility and fura-2 ratios determined using an IonOptix system modified for fluorescence analysis.

Analysis:

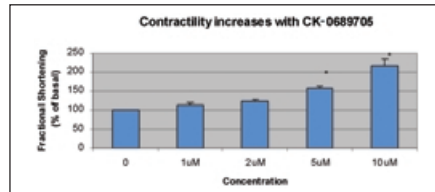
For each cell, ten or more contractility and calcium ratio transients at basal and after compound addition, were averaged and compared. Contractility average transients were analyzed using the IonWizard analysis program to determine changes in diastolic length, and fractional shortening (% decrease in the diastolic length; FS). For FS, data are normalized to basal values (basal equals 100%) and expressed as FS (% 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 (T75). Statistics were performed using the Students T-test or ANOVA as appropriate.

Reagents:

CK-0689705 provided by the Cytokinetics Chemistry department. All other reagents are from Sigma.

RESULTS

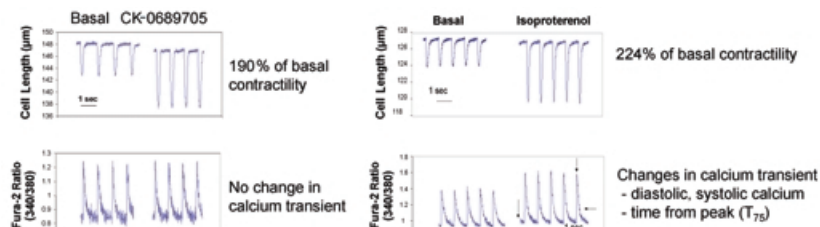
1. CK-0689705 increases myocyte fractional shortening



CK-0689705 increases cardiac myocyte contractility in a dose dependent manner. Contractility parameters are shown below.

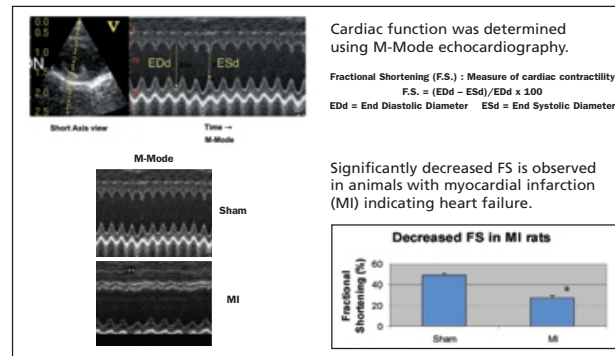
	Basal	1uM CK-0689705	2uM CK-0689705	5uM CK-0689705	10uM CK-0689705
Fractional shortening (% of diastolic length)	4.8 +/- 0.4	5.8 +/- 0.5	5.9 +/- 0.5	7.5 +/- 0.7*	10.2 +/- 1.0*
Fractional shortening (% of basal)	100	115 +/- 2.9	127.7 +/- 3.9	156.3 +/- 6.8*	215.9 +/- 19.1*
Amplitude of cell shortening (um)	7.1 +/- 0.6	8.2 +/- 0.8	8.7 +/- 0.8	10.9 +/- 1*	14.4 +/- 1.2*
Contraction velocity (um/sec)	-160.4 +/- 16.7	-170.0 +/- 17.9	-175.3 +/- 20.37	-186.5 +/- 20.7	-219.7 +/- 28.1
Relaxation velocity (um/sec)	138.7 +/- 18.0	152.4 +/- 19.9	156.7 +/- 21.3	183.4 +/- 24.3	236.1 +/- 31.7*

2. CK-0689705 does not alter the calcium transient



Individual tracings demonstrating that the observed increases in contractility are not due to increasing the calcium transient. The β -adrenergic agonist isoproterenol is shown for reference.

3. Identification of heart failure animals

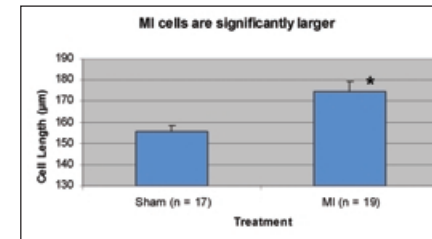


Cardiac function was determined using M-Mode echocardiography.

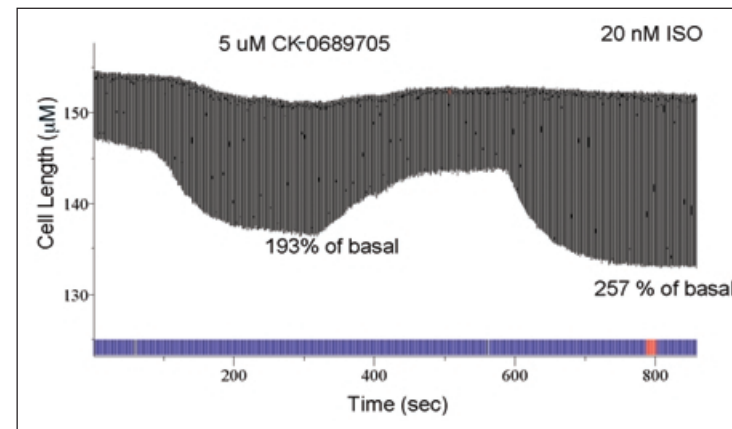
Fractional Shortening (F.S.) : Measure of cardiac contractility
 $F.S. = (EDd - ESd) / EDd \times 100$
EDd = End Diastolic Diameter ESd = End Systolic Diameter

Significantly decreased FS is observed in animals with myocardial infarction (MI) indicating heart failure.

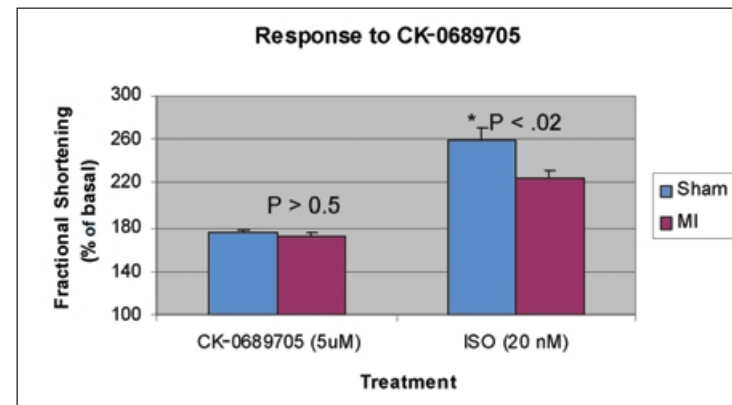
4. Cells are larger from MI animals indicating cellular hypertrophy, a characteristic of heart failure



5. Cells from MI rats respond equally well to CK-0689705, but have a truncated response to isoproterenol



Individual myocyte tracing (cell from sham animal) demonstrating increased contractility to challenge with CK-0689705 and ISO.



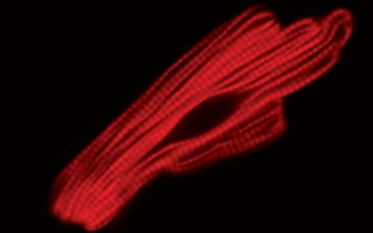
Equivalent increases in contractility are observed with the myosin activator CK-0689705 in cells from animals with defined heart failure.

However, due to β -adrenergic constant stimulation in heart failure, desensitization occurs and the response to ISO is significantly truncated.

REFERENCES

Cohn JN, Goldstein SO, Greenberg BH, Lorell BH, Bourge RC, Jaski BE, Gottlieb SO, McGrew F 3rd, DeMets DL, White BG. A dose-dependent increase in mortality with vesnarinone among patients with severe heart failure. Vesnarinone Trial Investigators. *N Engl J Med*. 1998;339:1810-1816.

Packer M, Carver JR, Rodeheffer RJ, et al. Effect of oral milrinone on mortality in severe chronic heart failure. *N Engl J Med*. 1991;325:1468-1475.



SUMMARY/CONCLUSION

CK-0689705, a direct acting myosin activator

- increases the fractional shortening in ventricular myocytes in a dose dependent manner
- does not increase the calcium transient
- increases contractility in cells from animals with defined heart failure

These results suggest that myosin activators such as CK-0689705 that increase cardiac contractility but do not alter the calcium transient may be useful therapeutics in the treatment of heart failure.