A hallmark of heart failure (HF) is decreased cardiac contractility. Previous efforts to increase contractility have included agents with direct β-adrenergic agonist, experimental, ISS, or indirect (calcium ionophore, neomycin, etc.) activity and have produced limited success. There is a need for agents that directly activate cardiac myosin. We have reported 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 systems. The cellular response to CK-0689705 is determined in adult rat ventricular myocytes isolated from Sprague-Dawley rats and cells with defined HF. Cellular contractility was measured using edge detection and myosin ATPase activities were measured using myosin ATPase assay.  During the HF phase, ventricular dysfunction is due to decreasing the calcium transient. In contrast, CK-0689705 significantly increased the calcium transient and contractility. Contractility was measured using fura-2 loaded myocytes.  Calcium transient increases induced by ISO with no further increase in the calcium transient, demonstrating CK-0689705 is not a β-adrenergic agonist. All experiments performed in duplicate. 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.

**Results**

1. CK-0689705 increases myocyte fractional shortening

![Graph showing CK-0689705 increases myocyte fractional shortening](image)

2. CK-0689705 does not alter the calcium transient

![Graph showing CK-0689705 does not alter the calcium transient](image)

3. Identification of heart failure animals

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.

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

![Graph showing cells are larger from MI animals](image)

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

![Graph showing cells from MI rats respond equally well to CK-0689705](image)

**Introduction**

Traditional inotropic agents used in treating acute decompensated heart failure increase cellular contractility by increasing the calcium transient. β-adrenergic agonist activate β-adrenergic receptors resulting in an increase in cAMP and activation of the PKA signaling cascade. Numerous proteins are phosphorylated including phosphodiesterase that results in an increase of the calcium transient and thus contractility. Phosphodiesterase (PDE) inhibitors increase the cAMP concentration by slowing CAMP degradation also resulting in an increase in contractility. 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 ATPases in the cardiac sarcomeres. 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**

- **Sprague-Dawley rats** were used in all experiments. For characterization of ISO responsiveness, only from 275-325 g normal rats were utilized. For MI experiments, cells had the chest opened and the left coronary artery ligated, resulting in anterior wall myocardial infarction (MI); for sham treatment, cells had the chest opened, exposed but not ligated. Sham and MI animals were 8 weeks post surgery.

**Cellular responses of the myosin activator CK-0689705 in normal and heart failure models**

ABSTRACT

**Abstract #1501**

Cytokinetics, Inc. South San Francisco, CA

**Introduction**

Myocardial infarction (MI) is the leading cause of death in the US and increased heart size (hypertrophy). Myocytes from HF rats had significantly truncated ventricular function as determined by echocardiography and increased heart size (hypertrophy). Myocytes from HF rats had significantly truncated ventricular function as determined by echocardiography. Cardiac function was determined using M-Mode echocardiography. Cardiac function (fractional shortening) of MI and sham anesthetized rats was determined by echocardiography with a GE System V and 10 MHz probe.

**Methods**

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

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

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**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 may increase cardiac contractility but do not alter the calcium transient may be useful therapeutics in the treatment of heart failure.