The Cardiac Myosin Activator, CK1122534, Increases Contractility in Adult Cardiac Myocytes But Does Not Affect the Calcium Transient or Depend on Second Messenger Signaling

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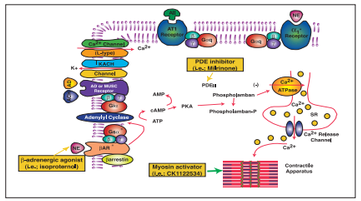
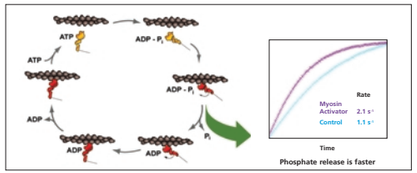
Abstract 2256

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 (bottom panel). 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.



Objective

Determine cardiac myocyte responses to, and mechanism of action of, a biochemically identified myosin activator, CK1122534.

Results

1. CK1122534 increases myocyte fractional shortening

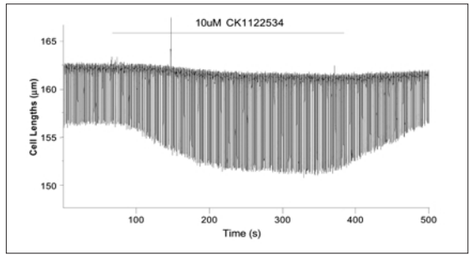


Figure 1: Individual tracing (above) and group data (below) of contractility parameters with CK1122534 demonstrating a significant increase in fractional shortening.

	Basal	2uM CK1122534	5uM CK1122534	10uM CK1122534	15uM CK1122534
Fractional shortening (% of basal)	100	118.46 +/- 4.23	135.56 +/- 4.15 *	152.32 +/- 6.09 *	159.44 +/- 5.57 *
Fractional shortening (% of diastolic length)	4.91 +/- 0.38	5.81 +/- 0.47	6.66 +/- 0.55 *	7.53 +/- 0.79 *	7.86 +/- 0.77 *
Amplitude of cell shortening (um)	6.43 +/- 0.65	7.58 +/- 0.82	8.60 +/- 0.91	9.66 +/- 1.17 *	9.95 +/- 1.09 *
Time to peak shortening (ms)	100 +/- 1	120 +/- 1	140 +/- 1 *	150 +/- 1 *	160 +/- 1 *
Time to 50% relengthening (ms)	170 +/- 1	190 +/- 2	210 +/- 2 *	220 +/- 2 *	230 +/- 1 *
Time to 75% relengthening (ms)	190 +/- 2	210 +/- 2	230 +/- 2	250 +/- 2	260 +/- 1
Contraction velocity (um/sec)	-135.79 +/- 17.56	-145.2 +/- 17.54	-149.1 +/- 17.43	-155.61 +/- 20.41	-143.17 +/- 16.42
Relaxation velocity (um/sec)	88.58 +/- 12.14	104.21 +/- 15.28	115.94 +/- 17.11	139.46 +/- 26.77	136.47 +/- 21.61
% Diastolic length	100	99.41 +/- 0.13	98.59 +/- 0.31	97.83 +/- 0.38	96.91 +/- 0.43

2. CK1122534 does not alter the calcium transient

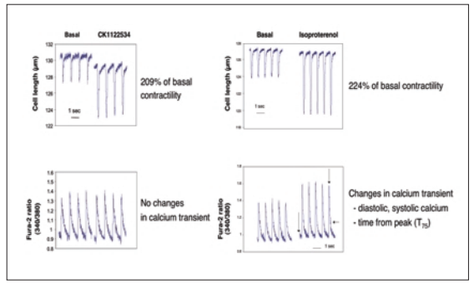


Figure 2: Individual tracings (above) and group data (below) showing contractility and calcium transients after isoproternol (ISO) and CK1122534 treatment. In contrast to ISO, CK1122534 treatment elicits a significant increase in contractility with no significant change in the fura-2 calcium ratio.

			Calcium Transient		
	n	FS (% of basal)	Diastolic Ratio	Systolic Ratio	Time to Baseline 75%
Basal	8	100	0.95 +/- 0.02	1.22 +/- 0.03	0.25 +/- 0.01
CK1122534		181.8 +/- 9.8	0.94 +/- 0.02	1.23 +/- 0.04	0.27 +/- 0.01
Basal	9	100	0.98 +/- 0.03	1.34 +/- 0.07	0.23 +/- 0.02
ISO		240.4 +/- 17.3	1.12 +/- 0.05 *	1.61 +/- 0.09 *	0.15 +/- 0.02 *

3. Propranolol (β-blocker) does not alter CK1122534 activity

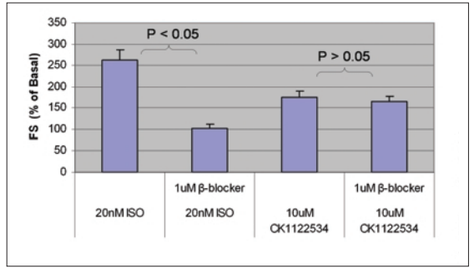


Figure 3: Treatment with the β-blocker, propranolol, completely inhibited the myocyte contractility response to 20 nM ISO. In contrast, myocyte contractility response to CK1122534 in the presence of 1 uM propranolol was unchanged.

4. CK1122534 lacks PDE activity

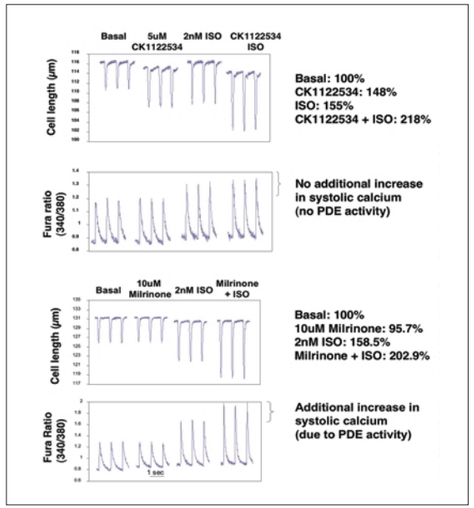


Figure 4: Individual tracings (above) and group data (below) showing contractility and calcium transient changes after treatment alone or in combination with CK1122534, ISO or Milrinone. Combination treatment with CK1122534 and ISO results in additive contractility but no additional increase in the calcium transient (no PDE activity). In contrast, the PDE inhibitor Milrinone in combination with ISO increases contractility and the calcium transient.

			Calcium Transient		
	n	FS (% of basal)	Diastolic Ratio	Systolic Ratio	Time to Baseline 75%
Basal	6	100%	0.93 +/- 0.01	1.26 +/- 0.01	0.26 +/- 0.03
5uM CK1122534		164.1 +/- 14.1	0.92 +/- 0.01	1.28 +/- 0.02	0.26 +/- 0.03
2nM ISO		226.7 +/- 33.2*	1.00 +/- 0.01*	1.47 +/- 0.04*	0.18 +/- 0.01*
5uM CK1122534 + 2nM ISO		294.6 +/- 40.7*	1.03 +/- 0.02*	1.52 +/- 0.04*	0.18 +/- 0.01*

Materials & Methods

Myocyte contractility experiments:
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 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.

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.

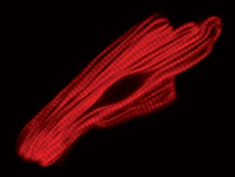
Analysis:
For each cell, ten or more contractility and calcium ratio transients at basal and after compound addition, were averaged and compared. Contractility average transients were analyzed using the IonWizard analysis program to determine changes in diastolic length, and fractional shortening (% 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:
CK1122534, a diarylurea, provided by the Cytokinetics Chemistry department. All other reagents are from Sigma.

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 Dec 17;339(25):1810-6.

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

CK1122534, a direct acting cardiac myosin activator:

- **increases the fractional shortening in ventricular myocytes in a dose dependent manner**
- **does not increase the calcium transient**
- **does not inhibit PDE activity**
- **is not affected by β-blockers**

These results suggest that cardiac myosin activators such as CK1122534, that increase cardiac contractility but do not alter the calcium transient, may be useful therapeutics in the treatment of heart failure.

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