The Cardiac Myosin Activator CK-1827452-Induced Myocyte Contractility is Unchanged in the Presence of β-Blockade

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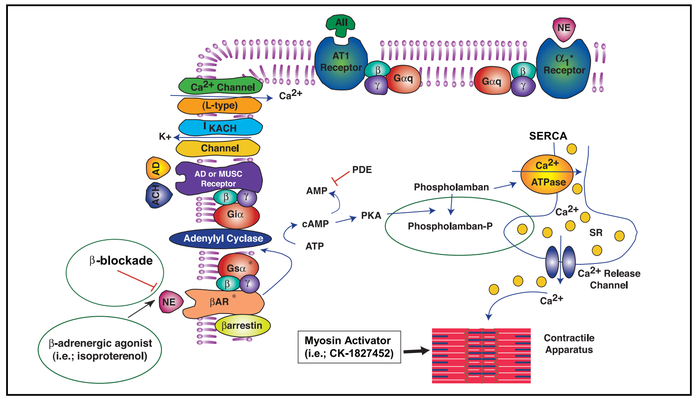
Introduction



We have previously reported on small molecule activators of cardiac myosin including CK-1827452, that increase myocyte contractility, not by increasing the calcium transient but by directly activating myosin in the cardiac sarcomere (ref 1-3). This mechanism may avoid the liabilities associated with the currently approved inotropic agents that are detrimental to patients with heart failure (ref 4-5).

The myosin activator, CK-1827452, is currently in Phase II clinical trials for the treatment of heart failure. As β-blockade is a cornerstone of heart failure therapy, it is clinically relevant to establish that a myosin activator can be used concurrently with β-blockers. Both propranolol and carvedilol are nonselective β-blockers while carvedilol is also an α1-adrenoreceptor antagonist. Additionally, at high dosages, carvedilol exerts calcium channel blocking activity and has significant antioxidant properties. Carvedilol was the first β-blocker labeled in the United States specifically for the treatment of heart failure.

Based on its novel mechanism of action, we reasoned that myosin activator-induced contractility should be unaffected by β-blockers, but have additive contractility with β-agonists. To test this hypothesis, and to further define the myosin activator mechanism of action, ventricular myocytes were isolated from adult Sprague Dawley rats and used in contractility experiments to determine cellular responses to myosin activators alone, and in combination with β-adrenergic agonists and antagonists. Additionally, the phosphorylation state of the calcium binding protein phospholamban (PLB) was determined.

These results demonstrate that, due to their novel MOA, myosin activators are independent of the β-adrenergic pathway. Importantly, CK-1827452-induced contractility is unchanged in the presence of β-blockade. These data suggest that circulating catecholamines or concomitant β-blocker use will not attenuate the effectiveness of myosin activators, such as CK-1827452, in the treatment of heart failure.

Methods

Myocyte contractility experiments: Myocytes were provided by the Cytokinetics Pharmacology department. 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 Tyrode's 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 and fractional shortening was monitored continuously. For combination experiments, each compound or combination of agents was perfused for 5 minutes in succession.

Cell Analysis: For each cell, ten or more contractility and calcium ratio transients at basal and 5 min after compound addition or combination treatment, 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.

Calcium transient experiments: Myocytes were loaded with 2 uM fura-2 AM (Molecular Probes) and simultaneous contractility and fura-2 ratios (340/380 nm; time to 75% of baseline (T75)) determined using an IonOptix system modified for fluorescence analysis. Calcium transients were analyzed using the IonWizard analysis program.

Western blots: Quiescent myocytes were incubated with compounds 15 min prior to addition of equal volumes of SDS loading buffer and boiling for 5 minutes. Resulting cell lysates were loaded onto an 18% Tris-Glysine gel and then transferred to a PVDF membrane using a dry transfer method (Invitrogen). Membranes were blocked in 5% milk and probed for phosphorylated phospholamban (P-PLB, 1:10,000; Badrilla) and GAPDH (1:2000; Cell Signaling). Experiments were repeated a minimum of three times and representative blots are shown.

Statistics: Data are mean +/- SEM. Statistics were performed using a one-way ANOVA with a post-hoc Student-Newman-Keuls test. $P < 0.05$ was considered significant.

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

Myosin activators increase cellular contractility without increasing the calcium transient

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 the myosin activator CK-1827452 (CK452, above) increases fractional shortening and the time to peak parameter (blue 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

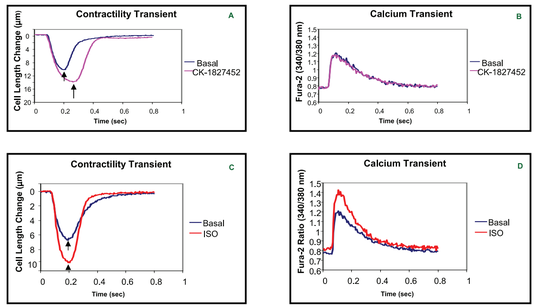


Figure 2. Representative tracings demonstrating that the myosin activator CK-1827452 (200 nM) increases cardiac myocyte contractility (A) without increasing the calcium transient (B). Arrows denote time of peak contraction. Note that myosin activators increase the time spent in contraction compared to basal. In contrast, the β-adrenergic agonist isoproterenol (ISO, 2 nM) shortens the time spent in contraction and increases contractility by increasing the Ca^{2+} transient (C and D, respectively).

In combination with β-adrenergic agonists, myosin activators demonstrate additive contractility with no further increase in the calcium transient

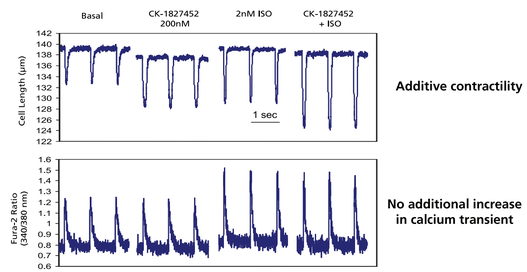


Figure 3. Representative tracings demonstrate that the myosin activator CK-1827452 has additive contractility with isoproterenol, but no additional increase in the calcium transient parameters. Group data are shown below.

Treatment	n	Fractional Shortening (% of basal)	Diastolic Ratio (340/380nm)	Systolic Ratio (340/380nm)	T75% (sec)
Basal	6	100 +/- 7.7	0.801 +/- 0.005	1.234 +/- 0.017	0.322 +/- 0.018
CK-1827452 (200 nM)		146.4 +/- 3.4*	0.783 +/- 0.004	1.192 +/- 0.015	0.331 +/- 0.025
ISO (2 nM)		168.7 +/- 12.9*	0.838 +/- 0.011*^	1.382 +/- 0.017*^	0.253 +/- 0.009*^
ISO + CK-1827452		211.5 +/- 14.5*^#	0.839 +/- 0.015*^	1.367 +/- 0.013*^	0.248 +/- 0.011*^

* P < 0.05 compared to basal
^ P < 0.05 compared to CK-1827452 alone
# P < 0.05 compared to ISO alone

In contrast to the β-adrenergic agonists, myosin activators do not increase the phosphorylation of phospholamban

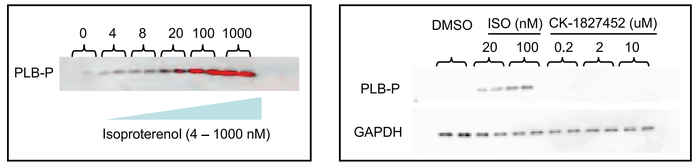
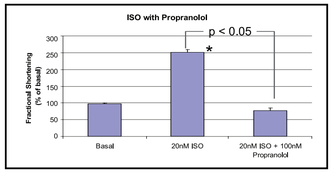


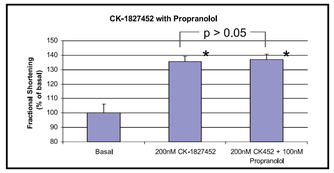
Figure 4. In quiescent myocytes, a dose dependent increase in phospholamban phosphorylation (PLB-P) is observed with isoproterenol treatment. In contrast, treatment with the myosin activator CK-1827452 does not increase phospholamban phosphorylation. GAPDH is shown as loading control. Representative blots shown.

In contrast to β-adrenergic agonists, contractility induced by the myosin activator CK-1827452 is unaffected by β-blockers

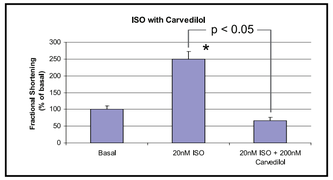
A Iso + Propranolol



B CK-1827452 + Propranolol



C Iso + Carvedilol



D CK-1827452 + Carvedilol

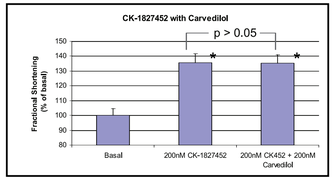
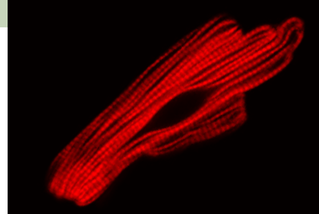


Figure 5. Treatment with the β-blockers propranolol (100 nM) or carvedilol (200 nM) obliterates ISO induced contractility (A and C) but has no effect on CK-1827452 induced contractility (B and D). The calcium transient fura-2 ratios and T75 (time to 75% of baseline) for the same cells (table below) are consistent with the myosin activator mechanism of action.

Treatment	n	Fractional Shortening (% of basal)	Diastolic Ratio (340/380nm)	Systolic Ratio (340/380nm)	T 75% (sec)
Basal	5	100 +/- 2.5	0.816 +/- 0.01	1.270 +/- 0.01	0.322 +/- 0.02
20nM ISO		258.8 +/- 9.9*	0.908 +/- 0.03*	1.906 +/- 0.09*	0.195 +/- 0.01*
20nM ISO + 100nM Propranolol		78.7 +/- 11.2^	0.769 +/- 0.02^	1.288 +/- 0.03^	0.282 +/- 0.01*^
Basal	6	100 +/- 6.2	0.826 +/- 0.02	1.288 +/- 0.02	0.336 +/- 0.03
200nM CK-1827452		135.4 +/- 4.5*	0.811 +/- 0.02	1.239 +/- 0.01	0.338 +/- 0.03
200nM CK452 + 100nM Propranolol		136.9 +/- 3.7*	0.779 +/- 0.03	1.207 +/- 0.03	0.367 +/- 0.03
Basal	6	100 +/- 10.5	0.793 +/- 0.01	1.257 +/- 0.01	0.337 +/- 0.02
20nM ISO		250.5 +/- 22.5*	0.882 +/- 0.04*	1.646 +/- 0.03*	0.186 +/- 0.01*
20nM ISO + 200nM Carvedilol		67.2 +/- 9.0^	0.770 +/- 0.02^	1.204 +/- 0.03^	0.290 +/- 0.03*
Basal	8	100 +/- 4.6	0.819 +/- 0.01	1.272 +/- 0.03	0.306 +/- 0.02
200nM CK-1827452		135.7 +/- 6.1*	0.775 +/- 0.02	1.235 +/- 0.02	0.337 +/- 0.02
200nM CK452 + 200nM Carvedilol		135.3 +/- 5.7*	0.775 +/- 0.02	1.219 +/- 0.03	0.309 +/- 0.01

* p < 0.05 compared to basal
^ p < 0.05 compared to ISO



Conclusions

The cardiac myosin activator CK-1827452

1) increases myocyte contractility by increasing the duration of the contraction with no increase in intracellular calcium.

2) in combination with the β-agonist isoproterenol, increases contractility with no further increase in the calcium transient.

3) in contrast to β-adrenergic agonist isoproterenol, does not result in the phosphorylation of the calcium altering protein phospholamban.

4) – induced contractility is unaffected by β-blockade.

These results demonstrate that, due to their novel MOA, myosin activators such as CK-1827452 are independent of the β-adrenergic pathway.

The data suggest that circulating catecholamines or concomitant β-blocker use will not attenuate the effectiveness of myosin activators such as CK-1827452 in heart failure. CK-1827452 is currently in Phase II clinical trials.

References

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