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 doses, carvedilol acts as a cardiac 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 or have additive contractility with β-agents. To test this hypothesis, and to further define the myosin activator mechanism of action, ventricular myocytes were isolated from adult male 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**

Myosin activator experiments: Myocytes were provided by the Cytokinetics Pharmacology department. Left ventricular myocytes were isolated from male rats using a collagenase digestion procedure and used within 5 hr of isolation. Myocytes were warmed to 37°C and perfused with Tyrode buffer and stimulated at 0.5 Hz. To determine contractility, myocytes were imaged through a 40x objective and field stimulated at 0.5 Hz. To determine cellular responses to myosin activators alone, and in combination with β-adrenergic agonists and antagonists, ventricular myocytes were isolated from adult male SD 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.

**CONCLUSIONS**

The cardiac myosin activator CK-1827452:

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

2) in combination with the β-blocker, carvedilol, 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 cardiac calcium 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 the treatment of heart failure. CK-1827452 is currently in Phase II clinical trial.

**REFERENCES**


2. Niu C, Anderson R, Cox D, Qian X, Morgan B, Malik F, Morgan B, et al. Quiescent myocytes were incubated with compounds 15 min prior to addition of equal volumes of 20 μM agonists or 20 μM agonists and compound. Resulting cell lysates were loaded onto an 8% Tris-Glysine gel and then transferred to a PVDF membrane using a dry transfer method. Membranes were blocked in 5% milk and probed for phosphorylated phospholamban (P-PLB, 1:10,000; Badrilla) and GAPDH (1:2000; Cell Analysis Inc, South San Francisco, CA) using monoclonal antibodies. Membranes were incubated overnight at 4°C and then incubated with horseradish peroxidase labeled secondary antibodies for 1 hour at room temperature. Membranes were washed in Tris buffer, blocked in 5% milk and probed for phosphorylated phospholamban (P-PLB, 1:10,000; Badrilla) and GAPDH (1:2000; Cell Analysis Inc, South San Francisco, CA) using monoclonal antibodies. Membranes were incubated overnight at 4°C and then incubated with horseradish peroxidase labeled secondary antibodies for 1 hour at room temperature. Membranes were washed in Tris buffer and then exposed to x-ray film. Membranes were analyzed using the IonWizard analysis program to determine changes in diastolic length, maximum contraction velocity, fractional shortening (% change in the diastolic length; FS) and time to peak. Data were averaged and compared. Contractility transients were analyzed using the IonWizard analysis program to determine changes in diastolic length, maximum contraction velocity, fractional shortening (% change in the diastolic length; FS) and time to peak.
