A Detailed Kinetic Analysis of the Cardiac Myosin Activator, CK-1213296, Suggests it Improves Cardiac Contractility by Accelerating Transition from the Weak to Strongly Bound States

Abstract #129

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Abstract

We have identified a small molecule activator, CK-1213296, of the cardiac myosin ATPase. This agent was characterized in steady state and transient kinetic assays to understand its mechanism of action.

CK-1213296 increases the steady-state rate of ATP hydrolysis of purified bovine cardiac myosin subfragment-1 (S1) and actin, as well as in bovine cardiac myofibrils, where the sarcomere structure is intact. We also find that CK-1213296 activates cardiac myosin selectively since no activation is observed in systems containing rabbit skeletal or chicken gizzard smooth muscle myosins. Analysis of the individual steps in the enzymatic cycle of cardiac myosin suggests that the actin stimulated release of phosphate is increased in the presence of CK-1213296. Additionally, we find that no other steps in the enzymatic cycle are affected by CK-1213296. Thus, the enzymatic step governing the weak to strong transition of S1 binding to actin is accelerated without affecting the release from the strongly bound state. This acceleration in the rate of transition from the weak to strong binding state appears to underlie its ability to increase force production in a dog model of cardiac function.

Introduction

Improving cardiac contractility by specifically activating cardiac myosin could offer the following potential advantages over current agents:

- No activation of second messenger signaling
- No increase in cardiac myocyte intracellular calcium
- No increase in heart rate
- No decrease in blood pressure

In addition, this mechanism of action is predicted to minimally impact cardiac oxygen consumption and thus potentially improve myocardial efficiency.

We sought to demonstrate the therapeutic hypothesis with the small molecule cardiac myosin activator, CK-1213296 (Figure 1). This class of molecules was identified in a high throughput screen for myosin activators, and here we describe details of their biochemical mechanism of action.

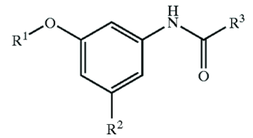
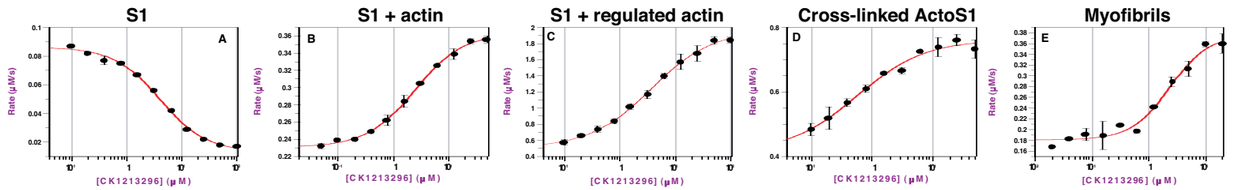


Figure 1. CK-1213296 is a member of a substituted benzamide family of myosin activators.

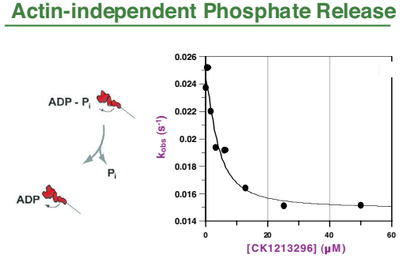
CK-1213296 is Active in Simple and Complex Systems



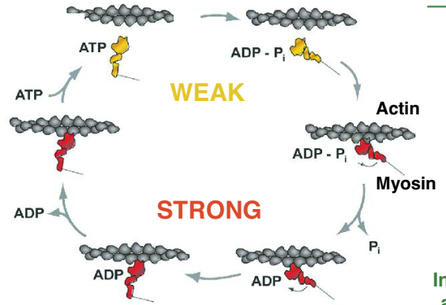
Dose responses of CK-1213296 in systems of increasing complexity: (A) bovine cardiac S1, (B) bovine cardiac S1 and bovine cardiac actin, (C) bovine cardiac S1 and calcium regulated actin at 50% of maximal calcium activation, (D) purified bovine cardiac S1 covalently attached to bovine cardiac actin, and (E) skinned bovine cardiac myofibrils at 50% of maximal calcium activation.

CK-1213296 Specifically Modulates the Weak -> Strong Transition

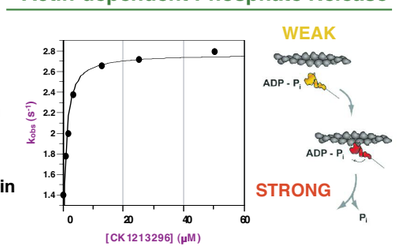
Actin-independent Phosphate Release



In the absence of actin, CK-1213296 inhibits phosphate release and thus non-productive ATP hydrolysis



Actin-dependent Phosphate Release



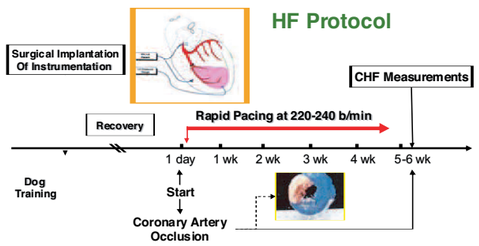
In the presence of actin, CK-1213296 accelerates phosphate release and thus productive ATP hydrolysis

Summary of transient kinetics

	DMSO Control	CK-1213296 (50 μM)
ATP binding to cS1	$1.5x10^6$ $M^{-1}s^{-1}$	$1.5x10^6$ $M^{-1}s^{-1}$
Myosin release from actin	$7.9x10^5$ $M^{-1}s^{-1}$	$7.9x10^5$ $M^{-1}s^{-1}$
ADP release from cS1	0.4 s^{-1}	0.4 s^{-1}
ADP release from acto-cS1	118 s^{-1}	127 s^{-1}

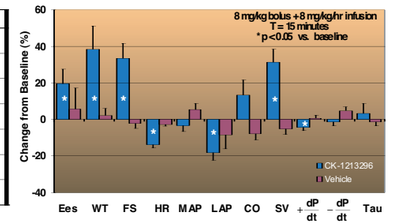
CK-1213296 Improves Cardiac Function in a Dog Model of Heart Failure

HF Protocol



Mongrel dogs are instrumented with pressure transducers in the left ventricle (LV), left atrium, and aorta. Ultrasound crystals are implanted for measurements of LV dimensions and wall thickness. In some dogs, a Transonic flow probe is implanted at the level of the ascending aorta for cardiac output or an occluder cuff is placed around the IVC to generate PV loops. Heart failure is induced by myocardial infarction using a coronary occluder around the left anterior descending artery followed by continuous rapid ventricular pacing (220-240 bpm) for several weeks. The pacer is turned off just prior to administration of CK-1213296.

	Start	CHF
HR (n=10)	108	134
MAP (n=9)	90	81
LAP (n=9)	4	24
dP/dt (n=10)	2761	1320
CO (n=4)	3.1	1.9
SV (n=4)	28	15
FS (n=10)	12.8	6.7



Conclusions

The small molecule CK-1213296 activates the actin-simulated ATPase of bovine cardiac myosin in systems with increasing complexity – from soluble actomyosin to skinned cardiac myofibrils.

CK-1213296 accelerates actin-dependent phosphate release from myosin in a dose-sensitive manner. This reaction, coupled to the weak to strong mechanical transition of myosin, suggests a mechanism for how the compound improves force production. In addition, CK-1213296 inhibits actin-independent phosphate release from myosin, reducing the rate of non-productive ATP hydrolysis.

Importantly, CK-1213296 has little effect on the other steps of the chemical cycle.

Administration of CK-1213296 increases contractility and improves cardiac function in a dog model of heart failure, suggesting that myosin activation could be a beneficial treatment for human heart failure.