The novel myotrope, AMG 594, is a small-molecule cardiac troponin activator that increases cardiac contractility in vitro and in vivo


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INTRODUCTION

Heart failure (HF) is the final pathway for many diseases that affect the heart. It is a clinical syndrome marked by impaired cardiac function and characterized by an imbalance between tissue demand and cardiac output (Rogler et al, 2012; Hilliker-Kleiner et al, 2006). Presently, there are approximately 5.7 million cases in the United States (Mozaffarian et al, 2016). HF requires lifelong treatment, and despite all available modalities available, morbidity and mortality remain high. Currently, all available cardiac therapies target only the compensatory neurohormonal cascades, without treating the primary pathophysiological problem in HF. Improvement in cardiac contractility through direct activation of the cardiac sarcomere is, thus, a potential strategy to treat HF.

RESULTS

ATPase Assays: Steady-state ATPase activity was measured using a pyruvate kinase and lactate dehydrogenase-coupled enzyme system as described in Hwee et al. (2015) and Malik et al. (2011). Non-myosin ATPase activity was subtracted from cardiac and slow skeletal myofibril assays in Figure 2 by subtracting the ATPase activity in the presence of a saturating concentration of the non-selective myosin II inhibitor (-) blasticystin.

METHODS

Animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals; all research protocols were approved by an Institutional Animal Care and Use Committee.

Preparation of Reagents: Myofilaments were prepared from flash-frozen bovine cardiac, bovine masseter, and rabbit psoas tissue as described in Hwee et al. (2015). Bovine cardiac myosin subfragment-1 (S1), actin, troponin, and tropomyosin were prepared as described in Malik et al. (2011). ATPase Assays: Steady-state ATPase activity was measured using a pyruvate kinase and lactate dehydrogenase-coupled enzyme system as described in Hwee et al. (2015) and Malik et al. (2011). Non-myosin ATPase activity was subtracted from cardiac and slow skeletal myofibril assays in Figure 2 by subtracting the ATPase activity in the presence of a saturating concentration of the non-selective myosin II inhibitor (-) blasticystin.

Skinned Cardiac Muscle Fiber Assays: Adult rat ventricular tissue bundles were skinned using 0.5% Brij 58 followed by storage in 50% glycerol as per Lynch and Faulker (1998). Muscle fibers were dissected from larger segments of tissue in rigor buffer at 4ºC (20 mM MOPS, 5 mM MgCl2, 120 mM potassium acetate, 1 mM EGTA, pH 7.0) and subsequently attached to a force transducer (403A, Aurora Scientific, Ontario, Canada) and a fixed post using 5% solution of methylcellulose in acetone. Isometric tension was measured at 10ºC in a relaxing buffer (20 mM MOPS, 5.5 mM MgCl2, 132 mM potassium acetate, 4.4 mM ATP, 22 mM creatine phosphate, 1 mg/ml creatine kinase, 1 mM DTT, 44 pM anti-foam, pH 7.0) supplemented with CaCl2 to produce appropriate concentrations of free Ca²⁺. Measurement of Cardiomyocyte Contractility and Calcium Transients: Adult rat ventricular cardiomyocytes were isolated and loaded with Fura-2 as described in Malik et al. (2011). Cardiomyocyte contractility and calcium transients were measured by edge-detection video microscopy and fluorescence photometry (IonOptix, Milton, MA) as described in Malik et al. (2011).

DISCUSSIONS

Study funding was provided by Amgen Inc and Cytokinetics, Inc. JDR, ASM, WS, LB, BR, EL, HYN, QZ, and JX are employees of Amgen Inc and hold Amgen stock options. JH, AR and FIM are employees of Cytokinetics Inc. and hold Cytokinetics stock options. KA, DSJ and AK are former employees of Amgen Inc.