Adult human primary cardiomyocyte-based platform for the profiling of positive inotropes with potential to treat heart failure

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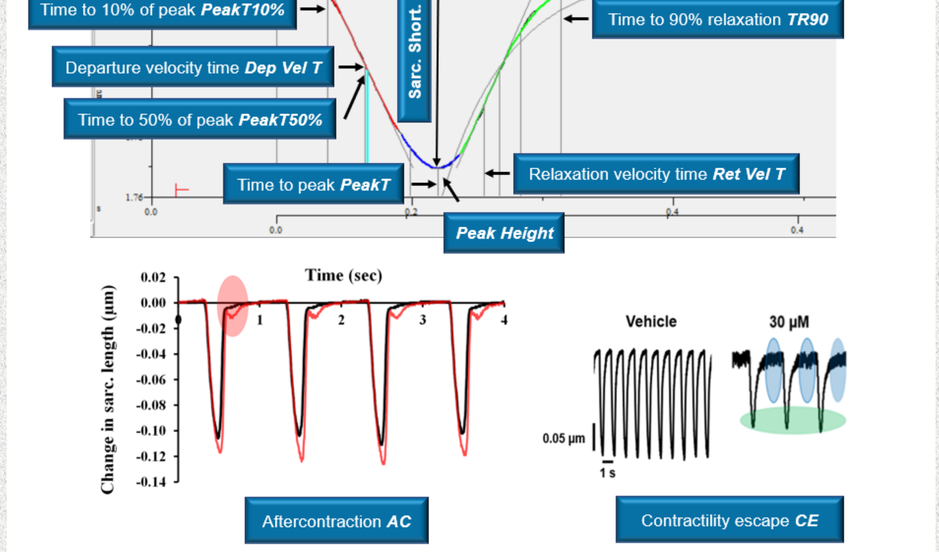
Introduction

Heart failure remains a major unmet medical need. Despite recent advances in the treatment of heart failure, in patients with reduced ejection fraction, high morbidity and mortality are not reduced by any treatment modality. From a therapy development standpoint, some key challenges originate from the incomplete understanding of the underlying pathophysiological mechanism and the lack of a relevant model to aid the selection of the best candidates for clinical development. The inability of current animal models to recapitulate all critical elements of the pathological state results in limited translation and high clinical attrition. Over the last few years, we have focused on the development of strategies and tools to bridge the translational gap by enabling large scale utilization of human primary cells and tissue. Access to cardiac tissue and cardiomyocytes from healthy as well as heart failure hearts, obtained from organ donors, allows for functional, biochemical- and omics-based investigation of the pathophysiology to a level unattainable in the past. To facilitate the identification of molecules with the most desirable efficacy profile, we developed a human cardiomyocyte contractility assay for the identification of positive inotropes with potential to correct contractility deficit in heart failure.

Methods and Selection of inotropes

Adult human primary ventricular myocytes isolated from ethically consented donor's hearts were used to measure fractional sarcomere shortening induced by field-stimulation and recorded using the IonOptix™ system. The stability of sarcomere shortening was assessed by continuous recording for 2 min. in Tyrode's solution with control vehicle (0.1% DMSO). The test articles were applied for a maximum of 250 s period or when a steady-state effect was achieved. Four to five ascending concentrations were tested. We modulated excitation-contraction coupling with a panel of well characterized inotropes (15 positives and 9 negatives) with diverse mechanisms of action.

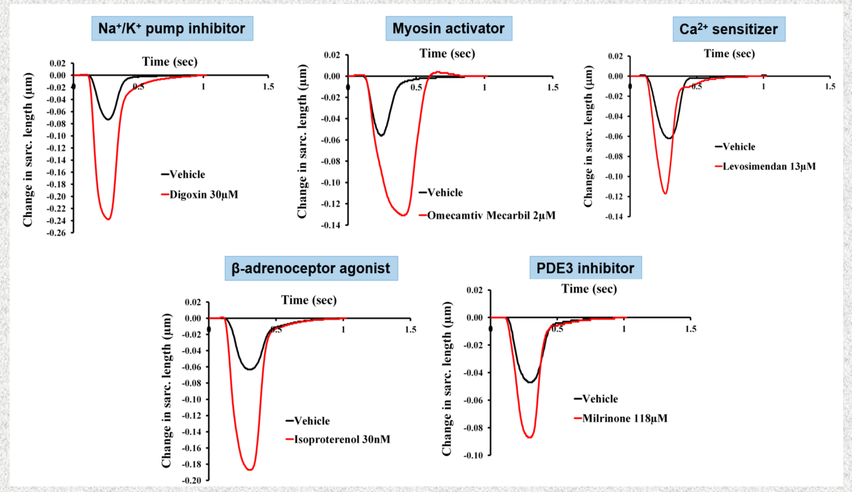
Parameters related to the contractility transient



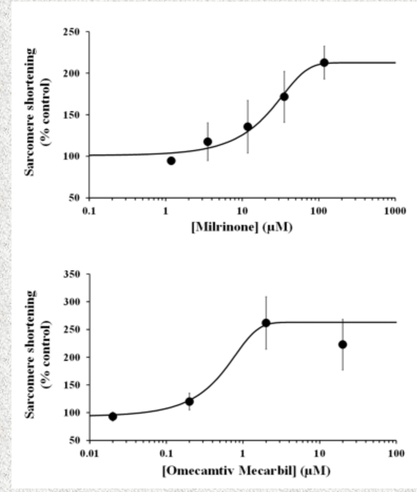
Modulation of excitation-contraction coupling with known positive and negative inotropes

Drug	Inotropic Effect	Mechanism of Action
Digoxin	Positive	Na^+/K^+ pump inhibition
Ouabain	Positive	Na^+/K^+ pump inhibition
SEA-0400	Positive	Na^+/Ca^{2+} exchanger inhibition
Omecamtiv Mecarbil	Positive	Myosin activation
Levosimendan	Positive	Ca^{2+} sensitization
Isoproterenol	Positive	Non-selective b-adrenoceptor activation
Epinephrine	Positive	Non-selective b-adrenoceptor activation
Dobutamine	Positive	b1-adrenoceptor activation
Milrinone	Positive	PDE3 inhibition
IBMX	Positive	PDE inhibition
Bay-K 8644	Positive	Ca^{2+} channel activation
Forskolin	Positive	Adenylyl cyclase activation
CaCl2	Positive	Hypercalcemia
N106	Positive	SERCA activation
Caffeine	Positive	RyR activation
Thapsigargin	Negative	SERCA inhibition
Ryanodine	Negative	RyR inhibition
Nitrendipine	Negative	Ca^{2+} channel inhibition
Nifedipine	Negative	Ca^{2+} channel inhibition
Diltiazem	Negative	Ca^{2+} channel inhibition
Mibefradil	Negative	Ca^{2+} channel inhibition
Verapamil	Negative	Ca^{2+} channel inhibition
Mexiletine	Negative	Na+ channel inhibition
Flecainide	Negative	Na+ channel inhibition

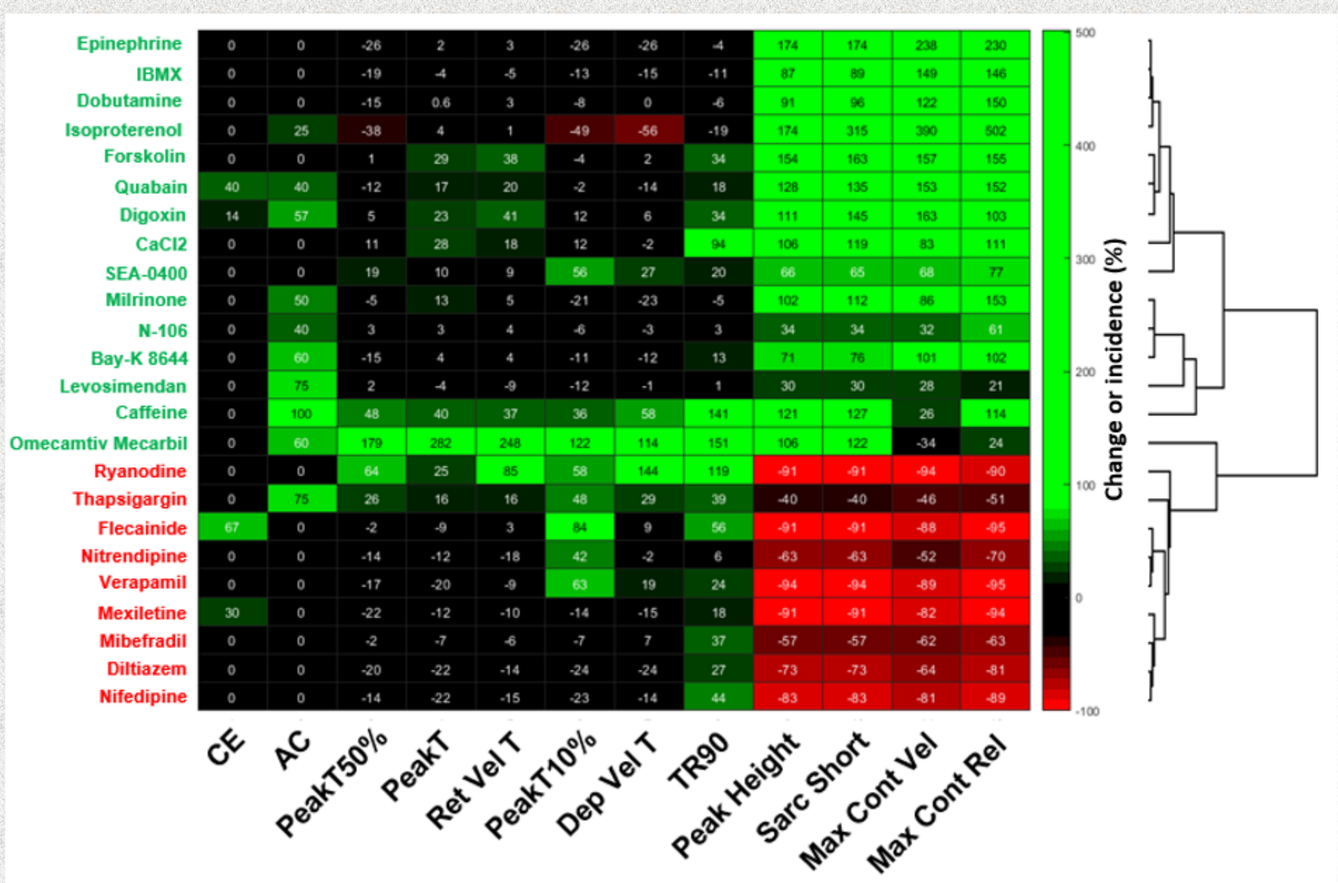
Identification of drug-induced positive inotropy



Concentration-effect curves

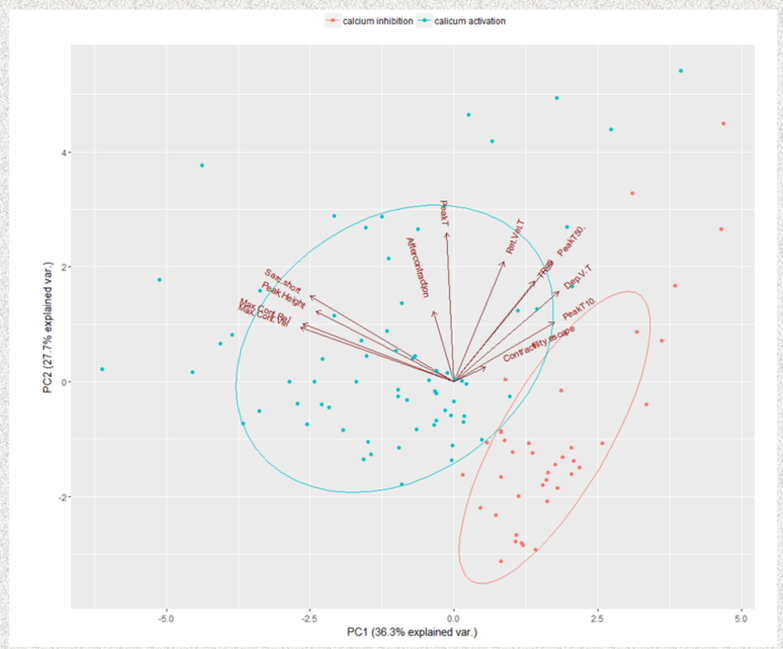


Segregation of different mechanisms for modulation of cardiac contractility: Hierarchical clustering



Red and green colors indicate decrease and increase of >25% and 10% change, respectively. Black colors indicate no effect (<-25% < % change < 10%). Numbers in boxes indicate means % change relative to vehicle, except for AC and CE (% incidence).

Segregation of different mechanisms for modulation of cardiac contractility: Principal component analysis



Segregation of compounds based on the ability to increase or decrease intracellular Ca^{2+}. Cluster borders mark the 70% confidence interval for predicting if a novel unknown compound induces a positive or negative inotropic effect via increasing or decreasing intracellular Ca^{2+}, respectively.

When considering the four negative inotropic mechanisms of action (Ca^{2+} channel, Na^+ channel, Ryanodine receptor or SERCA pump inhibition), the cloud borders mark the 70% confidence interval for predicting if a compound induces a negative inotropic effect via inhibition.

Summary

The adult human primary cardiomyocyte-based platform:

1. Can identify inotropic potential of molecules
2. Can enable the classification of inotropes in a mechanism-related mode
3. Will facilitate the identification of molecules with the most desirable pharmacological profile for the correction of specific forms of contractility deficit

Nguyen N et al., Front Physiol 8:1073. doi: 10.3389/fphys.2017.01073. eCollection 2017.