IonOptix MultiCell: High-Throughput Calcium and Contractility Acquisition in Left Ventricular Isolated Cardiomyocytes from Large and Small Animals

Keywords: Calcium, excitation-contraction coupling, isoproterenol

IonOptix System: MultiCell High Throughput System

Introduction

To date, high-throughput assessment of real-time isolated cardiomyocyte calcium and contractility has been unavailable to investigators seeking to improve throughput of isolated cells. In particular, the acute contractile effects of beta-adrenergic stimulants limit the ability of investigators to generate substantial paired cellular data sets. Beta-adrenergic stimulation accelerates cardiomyocyte excitation-contraction coupling (ECC, for reviews see [1–4]) kinetics through multiple pathways and can act as an experimental measure of cardiomyocyte functional reserve. Here we show a novel modality for high-throughput, repeated measures acquisition of isolated cardiomyocyte calcium and contractility prior to and following treatment with the beta adrenergic agonist isoproterenol.

Methods

Large Animal Cardiomyocyte Isolation

Cardiomyocytes were isolated from left ventricular midmyocardium of large animal hearts (see [5,6] for detailed protocol in porcine model). Following cardiectomy, left ventricular wedge preparations including a portion of the left anterior descending (LAD) coronary artery were excised and cannulated via LAD for retrograde coronary perfusion of liberase blendzyme. Ancillary coronary arteries were ligated to improve enzymatic penetration. After sufficient digestion, a portion of the midmyocardium was excised, immersed in bovine serum albumin (BSA) solution to halt enzymatic digestion, and cardiomyoyctes were mechanically dissociated from the tissue.

Murine Cardiomyocyte Isolation

Left ventricular cardiomyocytes were isolated from retrograde perfused langendorff hearts of mice.[7–10] Following cardiectomy, hearts were cannulated via the aorta to allow retrograde coronary perfusion of liberase blendzyme. After sufficient digestion, the heart was removed from the cannula and immersed in bovine serum albumin (BSA) solution to halt enzymatic digestion, and cardiomyoyctes were mechanically dissociated from the tissue.

Calcium Adaptation and Fura-2/AM Loading

Cardiomyocyte-containing solution was filtered through a 200 µm filter and slowly adapted to physiologic [Ca^{2+}] (2 mM) over 1 hour. During adaptation, cardiomyocytes were loaded with 5µM Fura-2/acetoxymethyl ester (Fura-2/AM) for ratiometric assessment of intracellular calcium concentration ([Ca^{2+}]$_i$).[11]

Adrenergic Responsiveness Protocol

Fura-2 loaded cardiomyocytes were moved to the IonOptix MultiCell stimulation chamber and equilibrated to physiologic temperature (i.e. 35-37° C) for 3 minutes prior to experimentation. Following equilibration, cells were electrically field stimulated at 1.0 Hz, 15 Volts while simultaneously assessing ratiometric [Ca^{2+}]$_i$ (Ex: 340 [Ca^{2+}-bound]/380 [Ca^{2+}-unbound] nm, Em: 510 nm) and contractility (fast fourier transform-derived sarcomere length) at 250 Hz for 10 seconds using the IonWizard software application. Following baseline acquisition under control conditions (*CTL*), cells were treated with 1 µM isoproterenol (*ISO*) to assess cardiomyocyte adrenergic functional kinetic reserve using the MultiCell repeated measures functionality.[12] Increased inotropic effects of isoproterenol resulted in gradual Ca^{2+} overload and spontaneous Ca^{2+} transients, therefore only electrically-stimulated (action-potential induced) transients prior to the advent of spontaneous activity were used for analysis.

Results

Multicell Permits Rapid, Paired Measurement of Murine and Large Animal Cardiomyocyte [Ca^{2+}]$_i$ and Contractility at Baseline and with Isoproterenol

Using the IonOptix MultiCell high-throughput repeated measures system, 34 murine and 91 large animal cardiomyocytes were assessed for systolic and diastolic intracellular calcium (**Figure 1** & **Figure 3**, respectively) and contractility (**Figure 2** & **Figure 4**, respectively) parameters. Data collection from each subject took <1 hour from start to finish and allowed pairwise statistical comparisons using the mark-and-find capability of the MultiCell High Throughput System. Systolic and diastolic parameters and kinetics were augmented in response to isoproterenol perfusion in both species. Suprisingly, given larger functionally viable cell yields, acquisition times from large animal isolations were significantly shorter than for murine models.

Discussion

The IonOptix Multicell High Throughput System newly empowers investigators to obtain high-throughput calcium and contractilty measurements for pairwise comparison in otherwise prohibitive conditions. Given the acute hypercontractility pursuant to beta-adrenergic perfusion, repeated measures assessment of contractility was not always possible, accounting for reduced sample in *ISO* conditions. However, this did not affect calcium measurments, which were consistent through multiple repeated measures (data not shown). This novel modality will allow investigators to expand investigations into cardiomcyte calcium and contractility beyond the limitations of current modalities.

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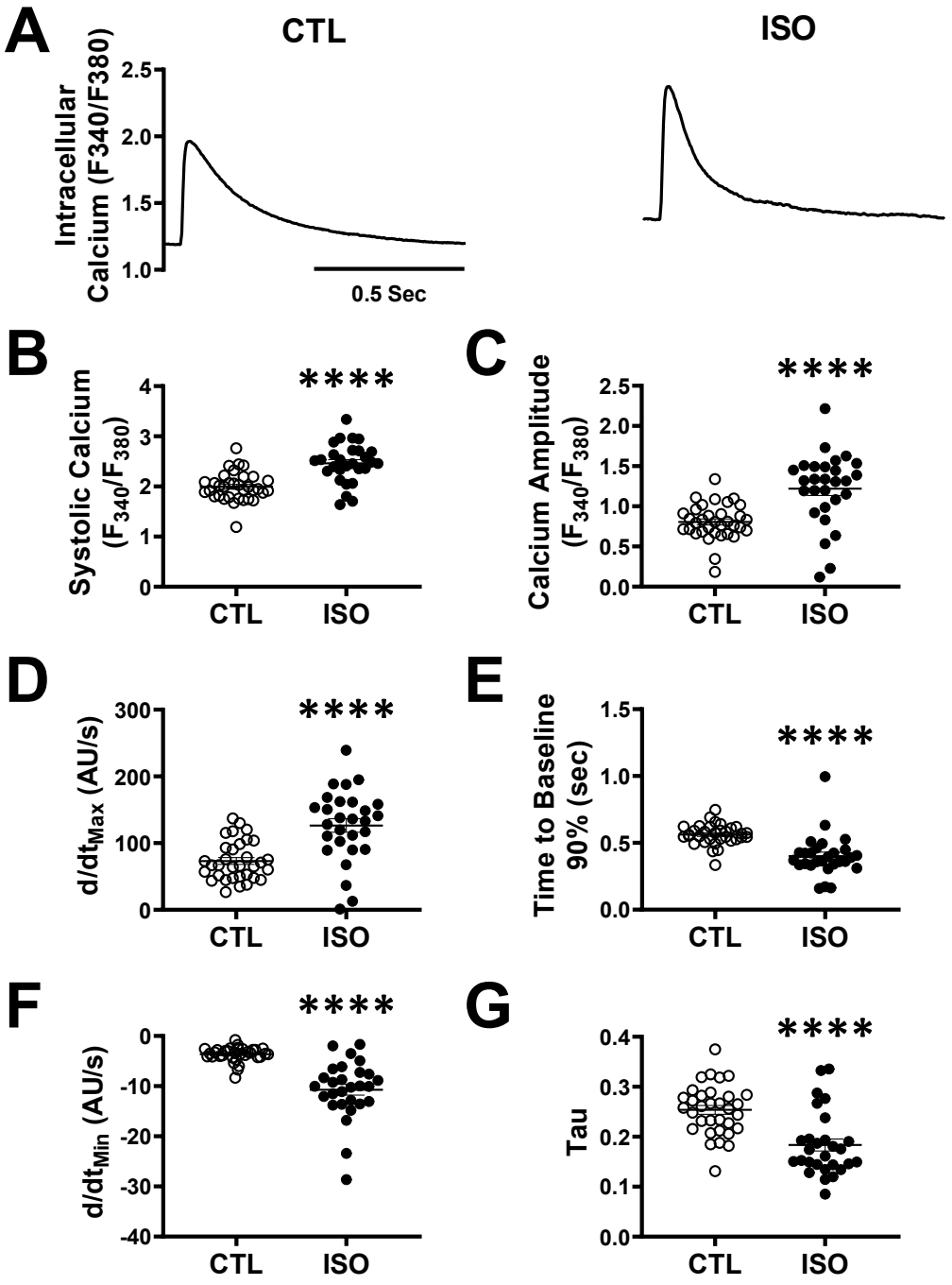

Figure 1. Isoproterenol Increases $[Ca^{2+}]_i$ and Kinetic Parameters in Murine Cardiomyocytes. (A) Average traces of $[Ca^{2+}]_i$ before (*CTL*) and after (*ISO*) 1 µM isoproterenol perfusion. Isoproterenol increased systolic $[Ca^{2+}]_i$ handling parameters **(B-C)** and kinetics **(D)**, while augmenting diastolic kinetics **(E-G)**. (N=1, n=34, ****P<0.001 vs. *CTL*).

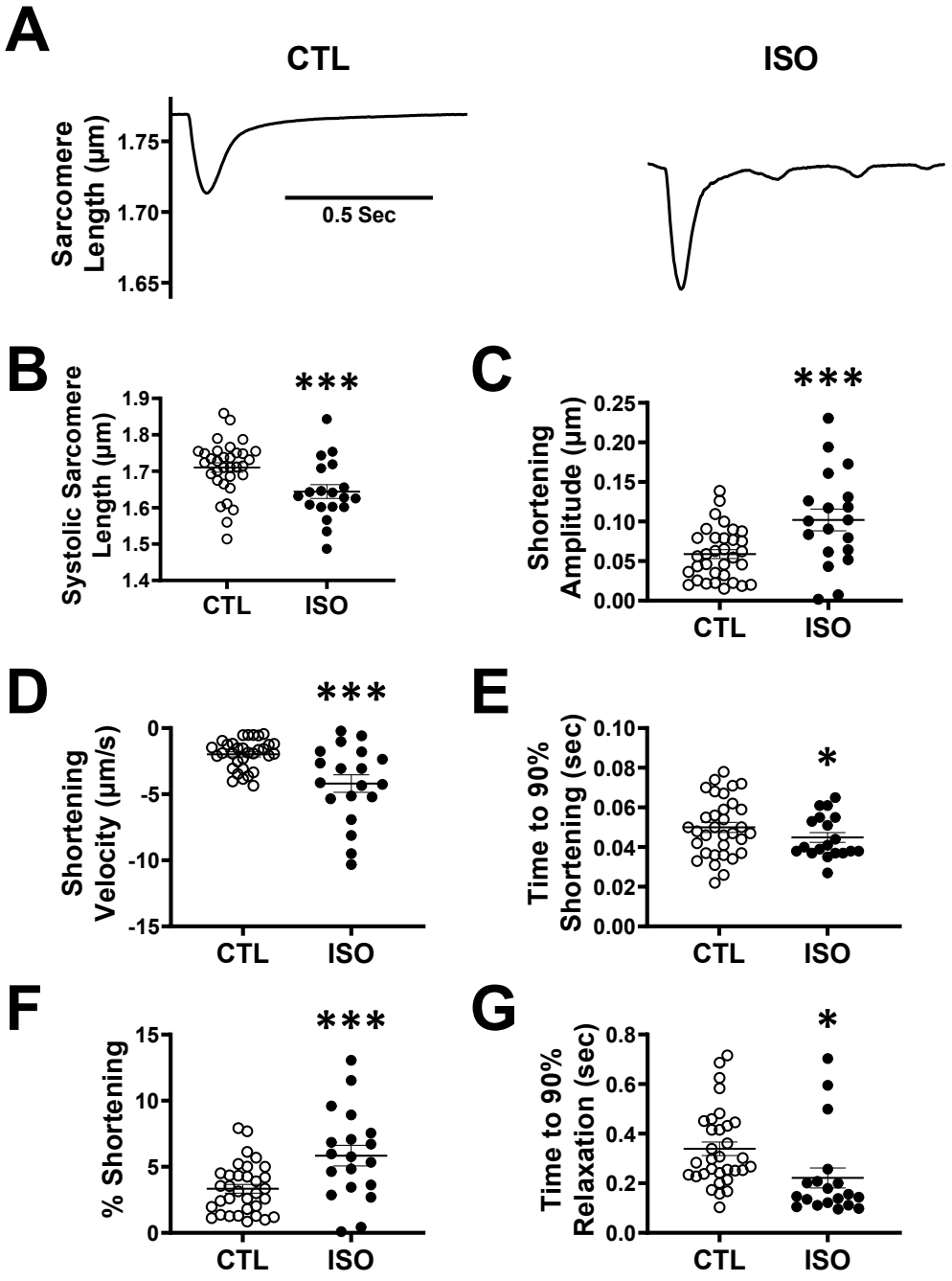

Figure 2. Isoproterenol Increases Cardiomyocyte Contractility and Contractile Kinetic Parameters in Murine Cardiomyocytes. (A) Average traces of cardiomyocyte sarcomere length before (*CTL*) and after (*ISO*) 1 µM isoproterenol perfusion. Systolic parameters of contractility showed increased contractility **(B-C, F)** and kinetic speed **(D-E)**, while relaxation speed was also increased **(G)**. (N=1, n=34, ***P<0.005 vs. *CTL*, *P<0.05 vs. *CTL*).

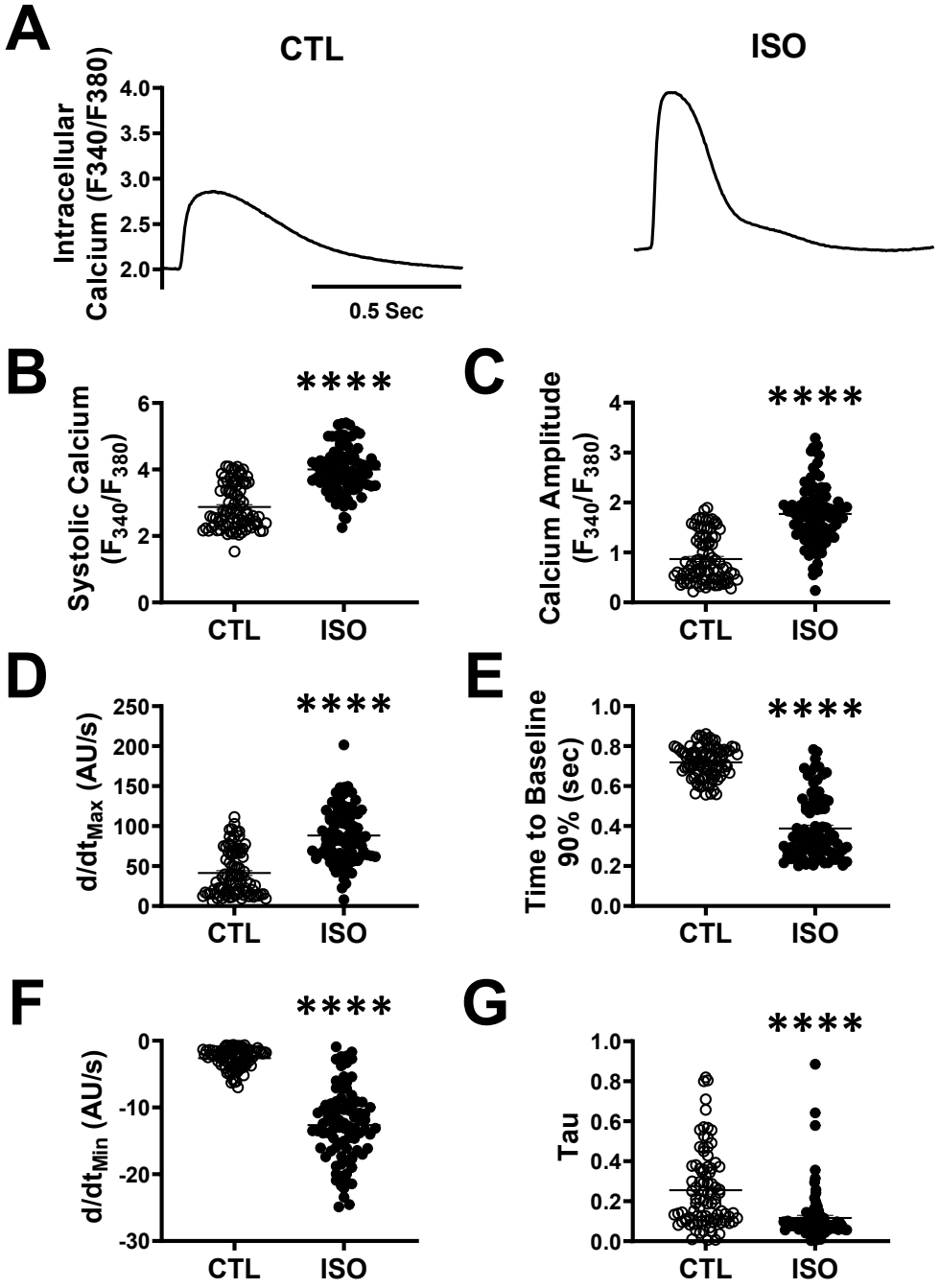

Figure 3. Isoproterenol Increases $[Ca^{2+}]_i$ and Kinetic Parameters in Large Animal Cardiomyocytes. (A) Average traces of $[Ca^{2+}]_i$ before (*CTL*) and after (*ISO*) 1 μM isoproterenol perfusion. Isoproterenol increased systolic $[Ca^{2+}]_i$ handling parameters **(B-C)** and kinetics **(D)**, while augmenting diastolic kinetics **(E-G)**. (N=2, n=91, ****P<0.001 vs. *CTL*).

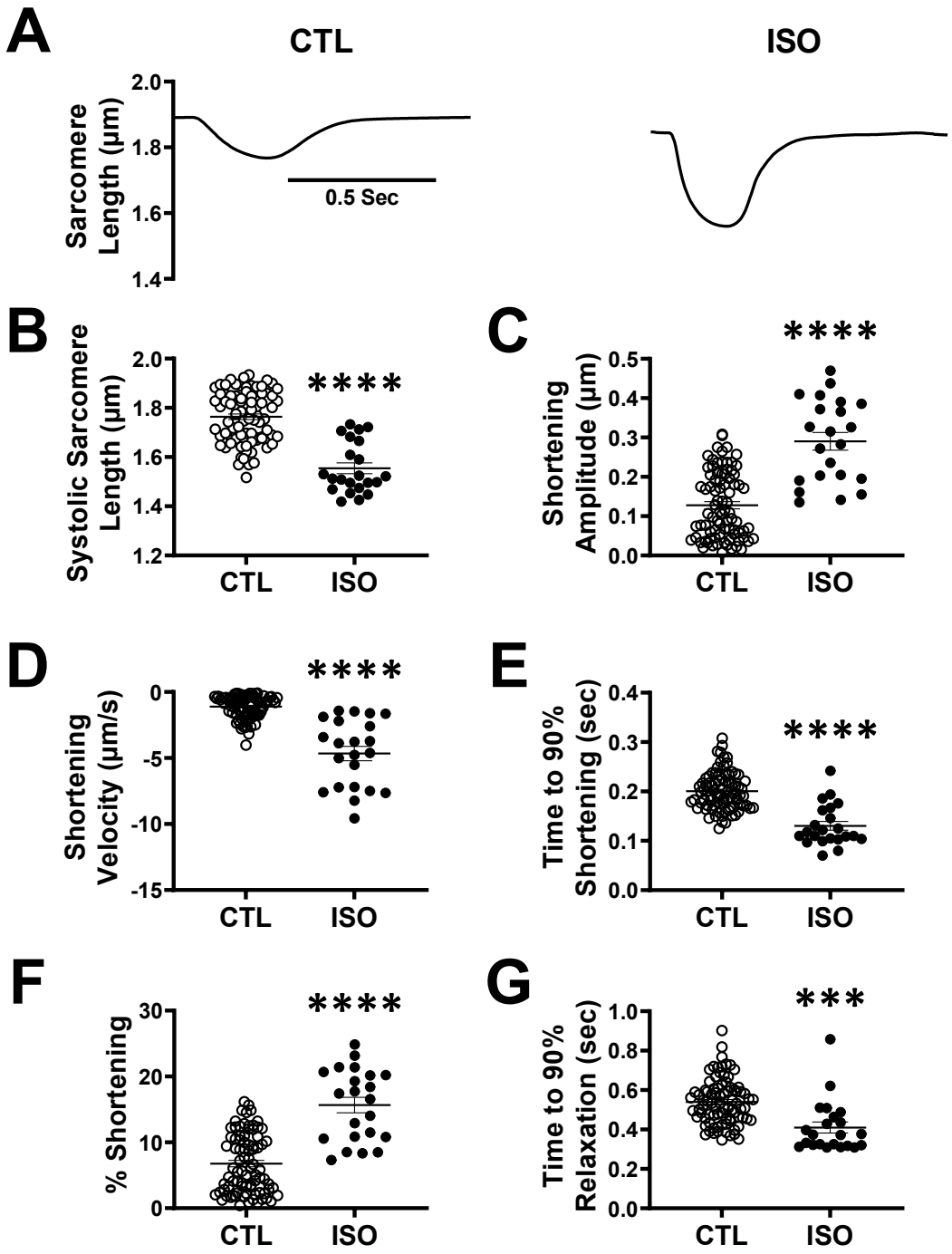

Figure 4. Isoproterenol Increases Cardiomyocyte Contractility and Contractile Kinetic Parameters in Large Animal Cardiomyocytes. (A) Average traces of cardiomyocyte sarcomere length before (*CTL*) and after (*ISO*) 1 µM isoproterenol perfusion. Systolic parameters of contractility showed increased contractility **(B-C, F)** and kinetic speed **(D-E)**, while relaxation speed was also increased **(G).** (N=2, n=91, ****P<0.001 vs. *CTL*, ***P<0.005 vs. *CTL*).

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