PHARMACOLOGICAL IDENTIFICATION OF AN L-TYPE CALCIUM CHANNEL IMPEDANCE SIGNAL IN HUMAN INDUCED PLURIPOTENT STEM-CELL DERIVED CARDIOMYOCYTES

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1 ABSTRACT

Impedance recordings of contractile human induced pluripotent stem-cell derived cardiomyocytes hold significant potential for drug discovery and cardiac safety given their easy implementation in medium throughput systems. However, the mechanisms underlying the complex impedance signal remain to be elucidated. The main component of the signal is the impedance twitch, a transient increase in electrode resistance that is obliterated by the myosin kinase inhibitor blebbistatin. Three additional small peaks (two initial negative deflections and one positive during relaxation) complete the complex impedance waveform. This work focuses on the pharmacological dissection of the first negative deflection that occurs after the sodium spike subsides and before the beginning of contraction. To test the hypothesis that the signal parallels the activation of calcium channels and not contraction, we investigated the effect of a calcium channel blocker (nifedipine), an agonist (FPL64176), and blebbistatin. Impedance recordings of iCell cardiomyocytes2 (CDI) were measured at 37°C using a CardioECR instrument (ACEA Biosciences) with pacing at 0.67 Hz. The impedance signal of iCell cardiomyocytes^2 shows two initial negative deflections of -0.38±0.02 Ω and -0.78±0.03 Ω followed by a 4.05±0.12 Ω positive peak (mean ± sem, n=20).FPL64176 at 10 and 30 nM increased the first negative peak amplitude by 14.9±4.2% (p>0.05) and 31.0±6.8% (p<0.05), respectively (paired t-test, n=4). In 10 µM blebbistatin FPL64176-activated peaks were smaller (10 nM: 8.8±2.8%, p<0.05) or not affected (30 nM: 1.0±3.7%, p>0.05). Nifedipine at 30 nM and 100 nM significantly decreased the first negative peak amplitude by 26.7±6.9% and 32.7±2.5% respectively, and no peak was observed in 10 µM blebbistatin. The data suggests that the first negative impedance deflection is determined by the opening of calcium channels and thus may be used as marker for excitation-contraction coupling.

2 INTRODUCTION

The combination of field potential and impedance recordings of stimulated or spontaneously beating stem-cell derived cardiomyocytes (SC-CM) hold a promising future in personalized medicine. However, the utility of this technology depends on the complete understanding of the mechanisms underlying the complex impedance waveform and its dependence on electrical excitability.

The main component of the signal is the impedance twitch (IT), a transient increase in electrode impedance that is obliterated by the myosin kinase inhibitor blebbistatin. Three additional small impedance deflections are detected, two preceding the impedance twitch (P1 and P2) and one occurring during relaxation (P3).

In this work we focused on P1, the first small transient impedance decrease that occurs after the fast sodium spike subsides and before contraction ensues. We hypothesized that P1 reflects events associated with excitation-contraction coupling and in particular L-type calcium channel activation.

Consistent with our hypothesis, P1 was decreased by the calcium channel blockers and increased by raising the extracellular calcium concentration or applying a calcium channel agonist. P1 was also increased in a SC-CM model of cardiac hypertrophy characterized by increased calcium channel activity.

We present evidence that P1 is part of a L-type channel associated impedance change (LCAIC) that is active during the excitation-contraction cycle.

3 METHODS

We used the xCELLigence RTCA CardioECR instrument (ACEA Biosciences) to record impedance and extracellular field potentials. iCell cardiomyocytes^2, myCell R403Q cardiomyocytes, a model of hypertrophic cardiomyopathy (HC) characterized by increased calcium currents in adult patients (Coppini et al. Circulation 127:575, 2013), and iCell cardiomyocyte controls (WT) were from Cellular Dynamics International/FUJIFILM. Twitch activity was recorded for spontaneously beating cells or field potential stimulated cells at frequencies allowed by the intrinsic spontaneous activity.

Analysis were performed using Origin, Matlab and macros written in VBA. The output of the instrument is the Cell Index, a measure of the electrode impedance relative to the background reading. During the analysis values were transformed to ohms according to Z=CI x 15Ω. Field potential and impedance twitches in each well were detected, time shifted relative to the negative peak of the sodium spike signal (t=0) and averaged. Single well averages where then combined in grand averages that summarize an specific treatment. Blebbistatin at 10 µM was used to remove motion dependent impedance changes while maintaining excitability.

4 CONCLUSIONS

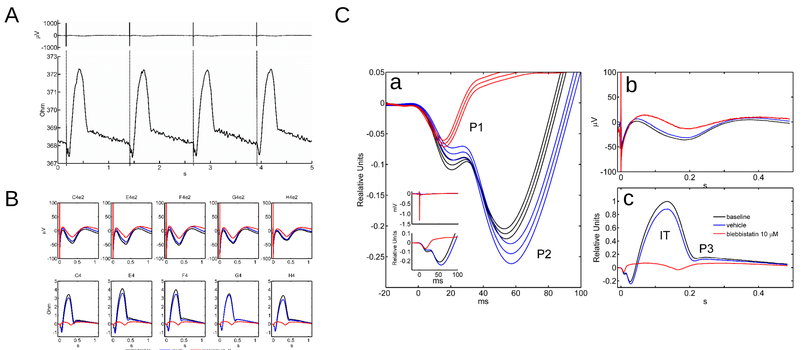
1) L-type calcium channel activation in SC-CMs is paralleled by an initial decrease in electrode impedance (P1) that amounts to ~10% of the impedance twitch amplitude.

2) P1 is the initial component of the L-type channel associated impedance change (LCAIC) unveiled when cell motion is inhibited by blebbistatin.

3) LCAIC is apparent before the onset of contraction and through the complete excitation-contraction cycle.

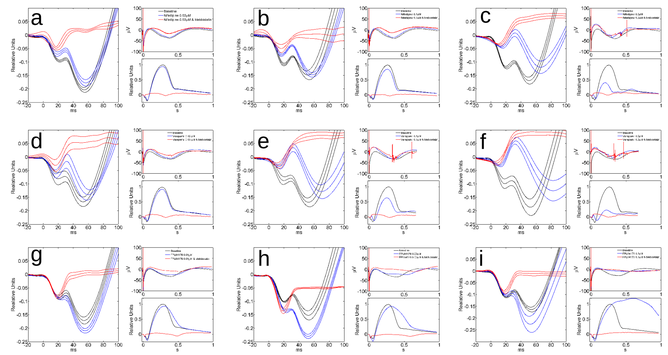
4) LCAIC depends on Ca^{2+} entry since it is magnified at larger extracellular calcium concentrations and during repolarization.

1) P1 follows excitation and precedes contraction



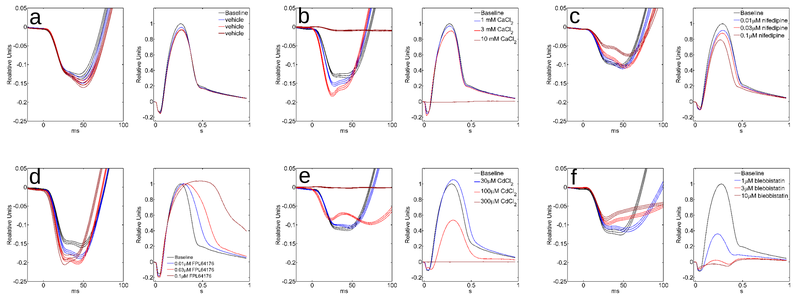
A) Field potential (top) and impedance signals (bottom). Vertical lines define the position of the negative peak of the sodium spike. Twitch activity was synchronized by field stimulation delivered at 0.8 Hz. Stimulus artefacts were removed for clarity. Note the two downward deflections that precede each twitch. B) Field potential and impedance local averages from five independent wells. Each average includes 47 Field potentials and twitches. Black, blue and red lines indicate signals recorded at baseline, after exposure to vehicle and in the presence of 10 µM blebbistatin respectively. C) Grand average of the four local averages shown at two different scales. To compare between wells local averages were normalized respect to the peak amplitude at baseline. Lines indicate mean ± SD. a) Magnification of P1 and P2. The inset emphasizes the location of P1 respect to the sodium spike. Note that P2 but not P1 are affected by blebbistatin. b) Field potential grand averages. c) Impedance twitch grand averages.

2) P1 is modified by L-Type calcium channel activators and inhibitors



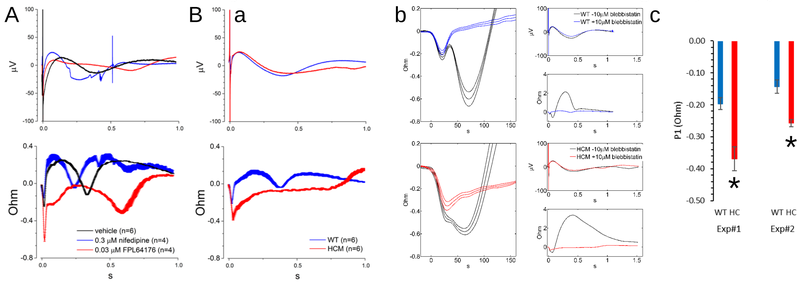
Grand averages of field potentials and impedance twitches activated by field stimulation delivered at 0.8 Hz. Lines in left panels indicate mean ± SD of four independent wells in each condition. Nifedipine (a, b, c) and verapamil (d, e, f) decreased P1 and the twitch amplitude and area in a concentration dependent manner. FPL64176 (g, h, i) increased the twitch area in a concentration dependent manner. P1 was affected in a biphasic manner showing a decrease at larger concentrations .

3) P1 is proportional to the Ca^{2+} driving force



Effect of increased extracellular Ca^{2+} concentrations on P1 (b). $CaCl_2$ concentrations indicated in the legend were added to the cultured medium containing 1.8 mM $CaCl_2$. No twitch activity and/or arrhythmic activity was recorded in 10 mM $CaCl_2$. Given the proximity of P1 and P2 (a) it was necessary to define the regions occupied by P1 using nifedipine (c), FPL64176 (d), Cd^{2+} (e) and blebbistatin (f). Drugs were added sequentially and the effect measure after 30 min exposure. n=7-8 for the different experimental conditions. Cells were field stimulated at 0.8 Hz.

4) P1 is part of the L-type channel associated impedance signal (LCAIC)



A) Field potential (top) and impedance twitch grand averages from cells exposed to 0.3 µM nifedipine/10 µM blebbistatin (blue lines), 0.03 µM FPL64176/10 µM blebbistatin (red lines) or vehicle/10 µM blebbistatin (black lines) from the experiments in Sections 1 and 2. Impedance signals are shown as mean ± sem. For comparison between treatments, impedance twitches in blebbistatin were subtracted from a baseline measured 32 ms before the stimulation artifact. Baselines in vehicle control (364 ±12 Ω), nifedipine (357±7Ω, p=0.642) and FPL64176 (396±19 Ω, p=0.210) were not significantly different. P1 in nifedipine (-0.15±0.02 Ω) and FPL64176 (-0.60± 0.05 Ω) were significantly different (p<0.01) than vehicle control (-0.28±0.02 Ω). Note the decrease in electrode resistance during repolarization.

Ba) Field potential (top) and impedance twitch grand averages from WT and HC SC-CM. Note the larger P1 signal in HC SC-CM consistent with the reported higher calcium current density (see Methods). b) Impedance twitch grand averages of WT SC-CM (top) and HC SC-CM (bottom). c) P1 values from the experiment shown in a and b where cells were plated at 25,000 cells/well (Exp#1) and a second experiment where cells were plated at 30,000 cells/well (Exp#2). In both experiments HC-SC-CMs show significantly larger P1 values (p<0.01).