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Highlighted Article

Excitation–contraction coupling in human heart failure examined by action potential clamp in rat cardiac myocytes

Patricia J. Cooper, Christian Soeller, Mark B. Cannell *

Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

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ABSTRACT

The effect of the loss of the notch in the human action potential (AP) during heart failure was examined by voltage clamping rat ventricular myocytes with human APs and recording intracellular Ca^{2+} with fluorescent dyes. Loss of the notch resulted in about a 50% reduction in the initial phase of the Ca^{2+} transient due to reduced ability of the L-type Ca^{2+} channel to trigger release. The failing human AP increased non-uniformity of cytosolic Ca^{2+}, with some cellular regions failing to elicit Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum. In addition, there was an increase in the occurrence of late Ca^{2+} sparks. Monte-Carlo simulations of spark activation by L-type Ca^{2+} current supported the idea that the decreased synchrony of Ca^{2+} spark production associated with the loss of the notch could be explained by reduced Ca^{2+} influx from open Ca^{2+} channels. We conclude that the notch of the AP is critical for efficient and synchronous EC coupling and that the loss of the notch will reduce the SR Ca^{2+} release in heart failure, without changes in (for example) SR Ca^{2+}-ATPase uptake.

1. Introduction

Heart failure is accompanied by changes in the ventricular action potential (AP) in both animal models and patients with heart failure [1]. Essentially, three phases are altered during the time course of the AP: (i) the amplitude of the fast upstroke is increased, (ii) the fast early repolarisation phase (or notch) is absent or diminished, and (iii) the AP is prolonged. The early effects (i and ii) appear to be related to a reduction in outward potassium currents I_{to} [2,3], while the prolongation of the AP may be additionally affected by a change in the inward rectifying potassium current I_{K1} [4]. The failing ventricular myocyte also often exhibits arrhythmogenesis due to early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs [1]) which may contribute to the development of ventricular tachyarrhythmias and lead to sudden cardiac death [5].

The reduced contraction strength of the failing heart has been linked to changes in both calcium (Ca^{2+}) handling [6–8] as well as changes in the contractile machinery itself [9,10]. Changes to proteins involved in Ca^{2+} handling (e.g. sarcoplasmic reticulum Ca^{2+}-ATPase, SERCA; phospholamban; ryanodine receptors, RyR; and Na^{+}–Ca^{2+} exchanger, NCX, but not generally the L-type Ca^{2+} channel (DHPR) have all been identified in various models of heart failure (for review see [11]). However, homeostatic mechanisms may limit the importance of some of these changes [12–14]. Complex interactions in Ca^{2+} homeostasis may make extrapolation of molecular changes to cell function difficult and, in any case, studying human heart function is complicated by difficulties in obtaining myocytes from a diseased tissue and it is even more difficult to obtain tissue from healthy humans.

Nevertheless, animal models have given useful insight into how complex signalling pathways may interact, but a problem arises from the extent to which AP changes in heart failure are mimicked in animal models. This difference could obscure, for example, the interaction(s) between the Ca^{2+} current (I_{Ca}) and SR Ca^{2+} release during normal and failing excitation–contraction (E–C) coupling. To address the problem we have examined E–C coupling in rat (and rabbit) myocytes clamped by AP waveforms derived from human tissue. The rationale for this approach resides in the fact that the rat expresses all the key E–C coupling proteins while the altered expression of surface membrane ion channels (that determine the AP time course) can be offset by current injection by voltage clamp. We are therefore able to examine the effects of the changed AP timecourse associated with human heart failure [15] on Ca^{2+} influx via DHPRs and the subsequent effect on calcium-induced calcium release (CICR) without the complication of other disease related effects. In a previous study, a decrease in the rate of early AP repolarization in rabbit was shown to depress SR Ca^{2+} release [16]. However, the complex interaction of the subsequent end-of-notch depolarization with I_{Ca} time course makes simple prediction of notch depth (voltage) changes on the magnitude of SR Ca^{2+} release

* Corresponding author.
E-mail address: m.cannell@auckland.ac.nz (M.B. Cannell).

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problematic. For example, the notch repolarization phase would mimic a faster I_{Ca} inactivation although the efffect is actually arising from a change in driving force rather than channel gating per se.

In this study, we focus on the importance of the loss of the notch of the human AP and its effects on I_{Ca} and Ca^{2+} transients, and show that the notch has profound effects on both the magnitude of Ca^{2+} release as well as the timecourse of the Ca^{2+} transient. It is notable that simply changing the AP timecourse to that of failing myocytes reproduces many of the changes reported for failing cardiac myocyte Ca^{2+} homeostasis despite the fact that no other changes were introduced (such as changes in SERCA function).

2. Materials and methods

2.1. Cell isolation and solutions

Wistar rats or NZ white rabbits were killed by lethal injection of sodium pentabarbitone (Rats: i.p. 140 mg/kg, $n=24$; Rabbits: i.v. 150 mg/kg, $n=3$) in accordance with the University of Auckland Animal Ethics Committee guidelines. The heart was rapidly removed and mounted on a Langendorf perfusion apparatus. Initially, the heart was perfused with a Ca^{2+}-free modified Tyrode solution containing (in mmol/L): 137 NaCl, 4 KCl, 1 $MgCl_2$, 10 HEPES, 10 glucose, pH 7.4 (with NaOH) for approximately 5 min. Ventricular myocytes were then enzymatically dissociated by perfusing the heart, for 10–13 min, with the modified Tyrode solution to which the following were added: collagenase (Worthington; 1.0 mg/mL), protease (Sigma Type I; 0.1 mg/mL) and 0.2 mmol/L $CaCl_2$.

Ventricular myocytes were voltage-clamped with the whole cell patch clamp technique. Electrodes (1.5–2.8 MΩ) were filled with a solution containing (in mmol/L): 120 Cs-aspartate, 10 HEPES, 5 MgATP, 0.001 cAMP, 10 tetraethylammonium (TEA) Cl, pH 7.3 with CsOH, and either 0.1 Indo-1 or 0.05 fluo-5F. Cells were superfused with a modified Tyrode solution containing: TEA-Cl (30 mmol/L) and CsCl (5 mmol/L) to inhibit K^+ currents and improve fidelity of the I_{Ca} recording. Nifedipine (10 μmol/L) and $CdCl_2$ (100 μmol/L) were used to measure background currents so that I_{Ca} could be identified by subtraction of records before and after exposure to these agents. Experiments were performed at 25 °C (confocal) or 35 °C as indicated.

Cell autofluorescence was measured after seal formation (before entering the whole cell configuration) and subtracted from all data recorded with indo-1. Fluorescence excitation was at 360 nm and fluorescence emission measured at 410 and 510 nm wavelengths and presented as the 410/510 ratio. In other experiments, myocytes were loaded with either fluo-4 AM or fluo-5F AM (both 5 μmol/L for 30 mins) and line scan images of Ca^{2+} transient morphology were recorded using a Zeiss 410 confocal microscope (488 nm excitation, >525 nm emission, expressed as F/F_0). Local calcium release events (similar to calcium spikes [17]) were measured in cells dialysed with EGTA (5 mmol/L) and fluo-5F (0.05 mmol/L) from the patch pipette.

2.2. Voltage clamp

Membrane current recordings were made using an Axopatch 200B amplifier and pClamp 9 software (Axon Instruments). The latter also controlled the indo-1 excitation light exposure period, or the confocal line scan acquisition. Human ventricular myocyte APs were digitized from published records of both control and failing myocytes [15], recorded at 37 °C, and 0.5 Hz repetition rate. These records were used as voltage commands for the AP clamp. The difference current, between control and after exposure to nifedipine or Cd^{2+} containing solution was normalized to cell capacitance and is defined as I_{Ca} (pA/pF). Each AP- or voltage-clamp waveform was preceded by at least three constant depolarizations, to standardize Ca^{2+} loading of the SR.

2.3. Statistics

Data are presented as mean ± SEM. Paired Student's *t*-test or ANOVA was used to test for significance where appropriate and resulting *P* values are given.

2.4. Computer simulation

A model describing stochastic DHPR and RyR gating calculated using the Monte-Carlo method, as previously described [18], was used to simulate the influence of human AP profiles on I_{Ca} and Ca^{2+} spark production. Briefly, a 12-state Markov model of DHPR gating was coupled into a dyad model and the action potentials were used to drive the DHPR gating model. To obtain the whole cell behaviour, 5000 dyad junctions or "couplons" [19] containing DHPRs and RyRs were simulated and whole cell current and average dyad cleft $[Ca^{2+}]$ time courses were calculated by summation of contributions from all couplons. The model parameters describing DHPR and RyR gating, and dyadic space properties were as in [18], however the model was modified to include the coupling of DHPR inactivation to cleft $[Ca^{2+}]$ and cytosolic $[Ca^{2+}]$ which was derived from experimental $[Ca^{2+}]$ records.

3. Results

3.1. Failing AP profile and Ca^{2+} transients

The normal human AP timecourse (N) shows characteristic spike and dome morphology but in the failing AP (F), the notch phase of repolarisation is absent and the AP duration is prolonged (see Fig. 1A, upper panel and [15]). A reduced peak I_{Ca} amplitude (Fig. 1A, middle panel, and Table 1) and slowed inactivation of I_{Ca} in response to the failure AP, was recorded. The slowed inactivation causes the integrated Ca^{2+} via I_{Ca} to gradually return toward near normal values (see Table 1, calculated over 400 ms). The Ca^{2+} transient recorded

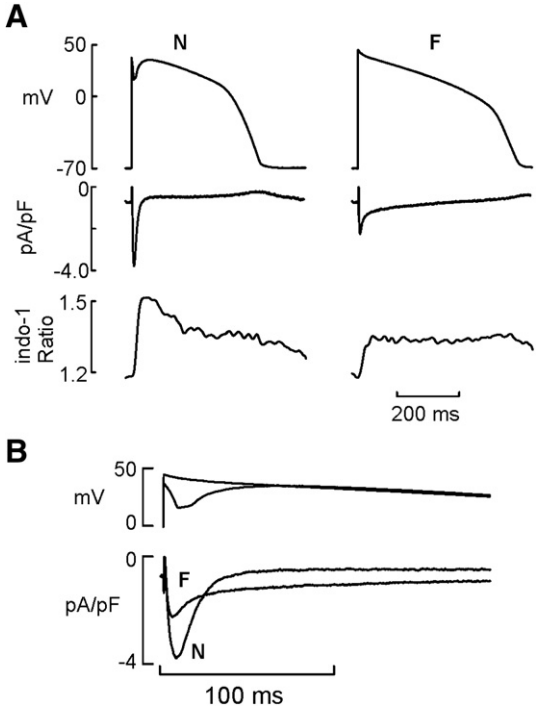

Fig. 1. Effect of action potential (AP) clamp of rat ventricular myocytes with human AP waveform on EC coupling. (A) Digitized human AP profiles recorded from non-failing (N) and failing (F) human myocytes (record from [15]; upper traces), and nifedipine-sensitive I_{Ca} (middle traces), and indo-1 fluorescence (lower traces). (B) Comparison of the I_{Ca} associated with the notch phase of the N and F APs.