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Original article

Electrical pacing counteracts intrinsic shortening of action potential duration of neonatal rat ventricular cells in culture

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Abstract

Previous studies have demonstrated the relationship between the functional electrophysiological properties of cultured neonatal rat ventricular myocytes (NRVMs) and the ability of the substrate to induce and sustain arrhythmia. The goal of this study was to examine the effects of chronic pacing at a constant rate akin to that in vivo, on the functional electrophysiological properties of NRVM monolayers. Confluent NRVM monolayers grown on 20 mm diameter cover slips were left either unpaced or were stimulated at 3 Hz for the duration of the culture, and were optically mapped on days 4, 6, or 8. Action potential duration at 80% repolarization (APD_{80}), conduction velocity (CV), and *Kv4.3* (I_{to}) and *NCX* protein expression were measured. The effects of the excitation–contraction uncoupler 2,3-butadione monoxime (BDM) were also investigated. The 2 Hz APD_{80} of non-paced monolayers decreased significantly on days 6 (137.1±13.9 ms) and 8 (109.8±9.0 ms) compared with day 4 (197.0±11.8 ms), while that of paced monolayers did not (206.8±9.7, 209.1±9.2, and 210.6±9.9 ms, respectively). The 2 Hz CV of non-paced monolayers increased significantly on days 6 (26.0±1.6 cm/s) and 8 (26.5±1.0 cm/s) compared with day 4 (20.0±1.0 cm/s), while that of paced monolayers did not change significantly (26.0±2.0, 26.0±1.0, and 23.8±1.2 cm/s, respectively). The restitution curves of APD_{80} and CV of paced monolayers were also unchanging from days 4 through 8. Despite the unchanging APD_{80} and CV, a decrease in *Kv4.3* expression and an increase in *NCX* expression were observed in paced compared with non-paced monolayers. Cessation of pacing or administration of BDM caused a reversal of phenotype back to that of non-paced monolayers. In summary, chronic electrical stimulation of confluent NRVM monolayers results in stabilization of APD_{80} and an advancement of the developmental rise of CV that is mediated by electromechanical coupling. These effects produce a steadier functional phenotype that may be beneficial for electrophysiological studies.

Keywords: Optical mapping; Chronic pacing; Neonatal rat ventricular myocyte; Monolayers; Cell culture

1. Introduction

Cardiac cell cultures are becoming an increasingly important and contemporary experimental system of minimal complexity that captures many of the salient features of myocardial tissue function, and are simple enough that tissue parameters can be controlled systematically. Primary cultures of neonatal rat ventricular myocytes (NRVMs) have been used to study intercellular communication [1], drug interaction [2], effects of passive and pulsatile stretch [3], and hypertrophy [4]. Monolayer sheets of NRVMs are a more electrophysiologically relevant preparation than isolated single cells, owing to the introduction of cell–cell communication and wave front propagation [5]. They constitute a versatile model system that has been used for the electrophysiological study of source-load effects in impulse conduction [6], ischemia reperfusion [7], gap junctional coupling and remodeling [3,8], functional anisotropy [9], anatomical reentry [10], spiral wave reentry [11,12], mixtures of cardiac and non-cardiac cells (including stem cells) [13–15], spatially discordant alternans [16], and gene expression of ion channels [17].

One complication regarding the use of this experimental model for electrophysiological studies is its changing phenotype over time. Studies of the in vivo temporal progression of cardiac myocyte development have demonstrated that cardiac

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cells transition from a hyperplasic to a hypertrophic state at 3 to 4 days following birth [18]. These changes are accompanied by a decreased action potential duration (APD) mediated by decreased hyperpolarization-activated current I_f [19], decreased sodium–calcium exchanger *NCX* [20], increased L-type calcium current $I_{Ca,L}$ [21], and increased transient outward current I_{to} [22]. In vitro NRVMs also exhibit developmental changes including increased cell size and morphology, altered protein expression patterns of the myocytes, and significant decrease in APD [22]. The in vitro electrophysiological developments of NRVMs have been linked to an upregulation of I_{to}, and are influenced by a downregulation in $I_{Ca,L}$ [22].

A key factor influencing the electrophysiological changes of in vitro model systems may be the contractile activity, which is generally spontaneous and uncontrolled during culture. Depending on how long it is measured after cell plating, the spontaneous beating rate of cultured NRVMs varies between 1 and 2 Hz [23] (a rate 3 to 4 times slower than the in vivo beating rate) and either becomes quiescent with increased I_k [24] and decreased I_f expression [19], or can exhibit power-law behavior in the presence of calcium current agonists [25]. APD is prolonged and ion channel expression (such as that of I_{to}) is altered in spontaneously beating myocytes compared with quiescent myocytes [22,26].

In this study, we used external electrical pacing to apply a fixed and sustained stimulation rate to NRVM monolayers in culture, and employed optical mapping to evaluate the hypothesis that the ensuing developmental changes in functional electrophysiology differ from that of non-paced monolayers, and perhaps follow a steadier pattern over time. Portions of this work have appeared in abstract form [27].

2. Methods and materials

All animals were treated according to protocols approved by the Animal Use and Care Committee at the Johns Hopkins University School of Medicine.

2.1. Cell culture

The ventricles of 2–3 day old Sprague–Dawley neonatal rat pups (Harlan, Indianapolis, IN) were excised, enzymatically dissociated with trypsin (US Biochemicals, Cleveland, OH) and collagenase (Worthington, Lakewood, NJ), and resuspended in M199 culture medium (Life Technologies, Rockville, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies) as described previously [9].

2.2. Stimulation setup

Electrical field pulses were used to stimulate the NRVMs by the method of Johnson et al. [28]. Rectangular-wave pulses, delivered by a stimulator (Grass S48, Astro-Med, West Warwick, RI), passed current via 0.25-inch diameter carbon electrodes (Ladd Research, Williston, VT) fully submersed in tissue culture medium and positioned at opposite ends of wells in an 8 rectangular-well culture plate (Fig. 1A). Up to 28 wells could be stimulated simultaneously. Toxic ionic and free radical buildup were minimized by supplementing the medium of non-paced and paced monolayers with 10 μM ascorbic acid as a reducing agent [28], changing the medium every 24 h and reversing the current direction of each pulse with a custom built polarity alternator. The stimulus threshold was determined by visual inspection of the cardiac cells and was consistent across

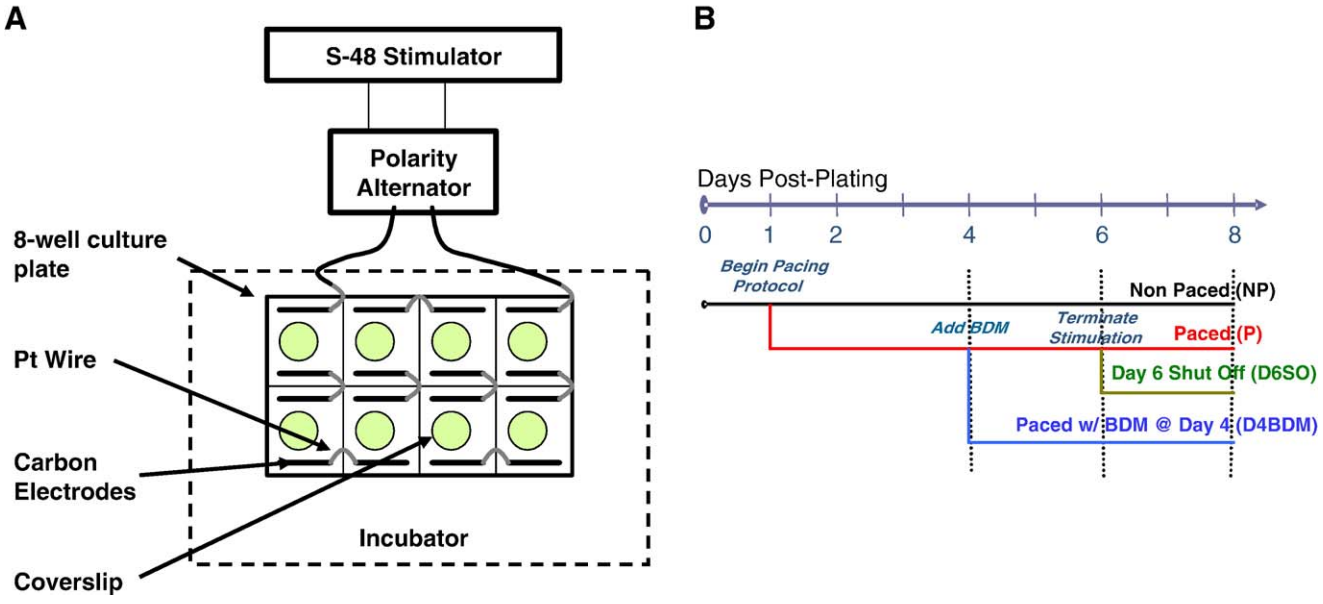

Fig. 1. Experimental setup and protocol. (A) Schematic of setup. Neonatal rat ventricular myocyte monolayers were plated in an 8-well culture plate with 1/8" diameter parallel carbon electrodes. The plate was placed in an incubator, and the carbon electrodes were connected to an external Grass S-48 stimulator via a polarity alternator. (B) Overview of experimental protocol. At 24 h post-plating, a subgroup of monolayers was paced at 3 Hz. At day 4, a subgroup of the paced monolayers was treated with 2,3-butanedione monoxime. At day 6, pacing was shut off in a final subgroup.