Differential effect of electrical stimulation and β-adrenergic stimulation on neonatal rat ventricular myocyte monolayer conduction properties

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Background: To develop novel treatments for cardiac arrhythmias, the electrical disturbances underpinning the arrhythmic phenotype in different settings requires more complete understanding. Multielectrode arrays (MEAs) provide a useful tool to non-invasively map cardiac electrophysiology in both cardiomyocyte monolayer cultures and atrial/ventricular tissue slices. Primary neonatal rat ventricular myocytes (NRVMs) represent an attractive model for studying inter-myocyte conduction properties *in vitro*, as cultures can be maintained beating spontaneously as a monolayer for 7+ days. Additionally, the capacity for short/long-term pharmacological treatment and/or gene manipulation (*e.g.* adenoviral infection) of NRVM cultures allows for investigation of the molecular mechanisms mediating inter-cardiomyocyte conduction. At present, there is limited characterisation data available regarding fundamental field potential and conduction properties in both spontaneously beating and electrically paced NRVMs.

Aim: The aim of this study was to characterize the basic electrophysiological properties of spontaneously beating and electrically-paced NRVM monolayers as a prelude to further studies assessing the molecular mechanisms of conduction heterogeneities.

Method: Hearts were removed from two-day old, anaesthetized (80% ethanol), neonatal Sprague-Dawley rats and cardiomyocytes isolated by collagenase and trypsin digestion. NRVMs (30,000 cardiomyocytes in 75 μl) were seeded onto the centre of fibronectin-coated (10 μg/ml) MEA chips (60PedotEcoMEA, MultiChannel Systems; electrode spacing 700 μm, diameter 100 μm, layout 8 × 8) and maintained in culture for 5-6 days. Field potentials were measured and conduction maps generated from spontaneously beating NRVMs at 37°C in the absence and presence of 1 μM isoproterenol using a filtered digital signal (high pass = 0.1 Hz, low pass = 3.5 kHz) with an MEA2100 system (MultiChannel Systems) sampling at 10 kHz. Paced electrical activity was recorded at 10% and 50% above the spontaneous beating rate using 2× capture threshold bipolar pulses (400 μs/phase) from the electrode nearest the spontaneous activity pacemaker. Results are presented as mean ± SEM. Comparisons between groups were performed with a Student's paired t-test or one-way ANOVA, as appropriate. Differences were considered significant at $P<0.05$.

Results: NRVMs were responsive to β-adrenergic stimulation, with spontaneous beating rate significantly increased in the presence of isoproterenol (basal *vs* isoproterenol-treated: 86 ± 6 bpm *vs* 144 ± 17 bpm, $n=3$, $P<0.05$). This was associated with a small, but significant increase in conduction velocity (23.37 ± 1.82 cm/s *vs* 24.92 ± 2.06 cm/s, $n=3$, $P<0.05$) and no change in field potential amplitude (3.17 ± 0.25 mV *vs* 3.09 ± 0.24 mV, $n=3$, $P=0.50$). In contrast, conduction velocity was significantly decreased in NRVMs electrically paced at rates 10% and 50% greater than basal (spontaneous beating rate *vs* spontaneous beating rate +10% *vs* spontaneous beating rate +50%, 24.22 ± 1.32 cm/s *vs* 18.07 ± 0.34 cm/s *vs* 18.73 ± 0.75 cm/s, $n=3$, $P<0.05$). Field potential amplitude remained unchanged, consistent with isoproterenol-treated NRVMs.

Conclusions: These preliminary findings showing NRVM responses to isoproterenol treatment and electrical stimulation, provide evidence to support the use of NRVMs as a suitable model for assessing cardiac conduction properties *in vitro*. The positive chronotropic response to isoproterenol treatment was associated with an increased conduction velocity, whereas electrical pacing was associated with decreased conduction velocity. This observation would be consistent with a β-adrenergic mediated phosphorylation of gap junction proteins, a mechanism which requires confirmation in the NRVM setting. This study demonstrates a first step in the validation of NRVMs as a model for assessing conduction properties in spontaneously beating cardiomyocyte cultures. Further studies will be performed to interrogate mechanisms underlying changes in cardiomyocyte conduction linked with arrhythmia vulnerability.