

## Multiple spontaneously active $\text{Ca}^{2+}$ waveforms in Nkx2.5-GFP cardiac lineage cells show selective modulation by $\text{I}_f$ channel blockade, endothelin I and elevation of intracellular cAMP

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**Introduction:** Isolated cells of the human and or mouse cardiac conduction system show location-dependent spontaneous activity, often described by changes in either electrical activity or in intracellular calcium ( $[\text{Ca}^{2+}]_i$ ) cycling.

**Aims:** To investigate the functional properties of spontaneously active cardiac lineage (Nkx2.5-eGFP<sup>+</sup>) cells derived from mouse embryonic stem cells.

**Methods:** Cardiomyocyte differentiation was initiated using the hanging drop method to generate embryoid bodies. Following 18-20 days of differentiation cell aggregates were dissociated and Nkx2.5-eGFP<sup>+</sup> cells isolated by FACS. Cells were loaded with the  $\text{Ca}^{2+}$  sensitive fluorophore, Fluo4-AM (10 $\mu\text{M}$ ), prior to high speed confocal imaging.  $\text{Ca}^{2+}$  fluorescence data were analysed for rate and changes in waveform kinetics (maximum changes in up and down slope and width at half height). Post-imaging, cells were fixed and immunolabelled with anti-PGP9.5 (a Purkinje cell marker).

**Results:** On the basis of frequency and width at least five distinct spontaneously active populations were present in the Nkx2.5-eGFP<sup>+</sup> cardiac lineage cells. The high frequency population were less sensitive to the funny channel blocker, ZD7288 (10 $\mu\text{M}$ ), but more sensitive to ryanodine (10 $\mu\text{M}$ ). Endothelin I (10nM) increased oscillation frequency, except in the slowest frequency waveforms. Angiotensin II (100nM) was without effect.

**Discussion:** Pacemaker automaticity mainly involves  $\text{I}_f$  current and/or ryanodine receptor mediated  $\text{Ca}^{2+}$  release from the SR. The slowest frequency cells were positive for PGP9.5, indicating that they are Purkinje-like cells. This study has shown that spontaneously active cells can be readily isolated from Nkx2.5-eGFP cardiac lineage cells. These cells are suitable for the investigation of the cardiac conduction system.