Circadian rhythm in pro-arrhythmic activity of pulmonary vein cardiomyocytes

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Introduction: Cardiomyocytes in the pulmonary vein (PV) sleeves are a major source of ectopic activity driving atrial fibrillation (AF) and episodes of AF in patients are more prevalent at night. While it is known that circadian clocks within the heart regionally control 24-hour variation in pacemaking and ventricular repolarisation, the mechanisms underlying the nighttime preponderance in AF are unknown.

Purpose: This study addresses the hypothesis that circadian rhythms exist in the pro-arrhythmic activity of PV cardiomyocytes.

Methods: Male Wistar rats maintained in a 24-hour cycle of 12-hr light/dark (lights-on at Zeitgeber time, ZT=0; lights-off, ZT12) were subject to terminal general anaesthesia (140 mg/kg Na pentobarbital i.p.) at ZT=0, 6, 12 or 18 hr and the hearts removed. RNA was extracted from left atrial (LA) appendage (LAA) and proximal PV at the LA/PV junction (3 rats per ZT) and RNA sequencing conducted. Reads were mapped to the rat genome, counts normalised and models in which ZT was or was not included as a factor compared (Likelihood Ratio Test, adjusted-P<0.01). Whole-cell current clamp recordings were made from cardiomyocytes isolated from the proximal PV (n=308) and the LAA (n=264) (N=74 rats). The effects of noradrenaline (NA, 1 µM) and acetylcholine (ACh, 1 µM) were examined. Data were plotted against the ZT of the time of recording and fitted to a sine wave to establish circadian rhythmicity (P<0.05, extra-sum-of-squares F-test). The effect of ≥24 hr constant dark in the period immediately before experiment was examined. Data are reported as mean ± standard error.

Results: The expression of 1368 genes varied significantly with ZT, including circadian clock components (e.g. Bmal1, Per1). PV cells were larger than LAA cells (73±1.6 pF vs 53±1.3 pF, P<0.0001) and had more depolarised resting membrane potential (-69±0.2 mV vs -72±0.1 mV, P<0.0001). Both cell types showed circadian variation in action potential duration at 90% repolarisation (APD90) and frequency of pro-arrhythmic activity. Pro-arrhythmic activity was greatest in PV cells and the frequency was greater during the rest phase (ZT0-12) in both cell types. In contrast, the circadian rhythm in APD90 differed between cell type, with the longest APD90 recorded during the active phase in PV cells (ZT12-24) but during the rest phase in LAA cells. Pro-arrhythmic activity was increased by NA and decreased by ACh in both cell types with maximal effect during the rest phase.

Conclusion: Circadian variation in APD90 and proarrhythmic-activity has been demonstrated in isolated proximal PV and LAA cardiomyocytes, with differences in rhythm between the two cell types. RNA sequencing suggests the presence of peripheral clocks in LAA and PV cardiomyocytes. Understanding these mechanisms is important in development of future therapeutic options for AF.