Circadian Pattern to Arrhythmias in a Genetic Mouse Model of Heart Failure

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Abstract

Arrhythmias and sudden cardiac death have a circadian pattern of occurrence in humans. We hypothesized that a circadian pattern of arrhythmias is also present in transgenic mice with cardiac-specific overexpression of tumor necrosis factor alpha, which develop a dilated cardiomyopathy, arrhythmias and heart failure. To test this hypothesis, telemetry monitors were implanted and 24hr, ambulatory ECG was recorded ≥ 5 days later. Animals were maintained on a 12hr light/dark cycle. The incidence of ventricular arrhythmias increased between 3pm and 12am, whereas atrial arrhythmias surged between 4am and 12pm. This circadian pattern of arrhythmias is in excellent agreement with human data (taking into account a 12-hour shift between activity periods in diurnal humans and nocturnal rodents). The molecular mechanisms underlying such circadian patterns of arrhythmias were examined using gene expression analysis and mathematical modeling.

1. Introduction

Arrhythmias have a well known circadian pattern of occurrence: the incidence of ventricular arrhythmias increases in the morning and afternoon, whereas atrial arrhythmias often occur at night. Transgenic and gene-targeted mouse models have been used to generate models of heart failure and as a tool to identify the molecular basis of cardiac ionic currents and arrhythmia mechanisms. However, it is not known whether a circadian pattern of arrhythmias is also present in mice. The molecular mechanism that defines the circadian pattern (or 'clock') of biochemical processes has been extensively studied in non-cardiac tissues. The 'clock' mechanism arises from 24-hour oscillations in the expression of specific 'clock' genes (including BMAL1, per2, and cry1), which are generated by reciprocal regulation of transcription and translation [1]. Such genes also control a variety of downstream physiologic processes, in particular, metabolic substrate utilization. The electrophysiological consequences of a circadian rhythmicity of gene expression in the heart are poorly understood. We hypothesized that anatomic heterogeneities of clock gene expression could occur in heart failure, creating spatial heterogeneities of cardiac physiologic processes, and providing a proarrhythmic substrate.

2. Methods

Telemetry ECG recording in a genetic mouse model of heart failure

Transgenic (TG) mice with cardiac-specific overexpression of tumor necrosis factor alpha (also referred to as TNF 1.6 mice previously) develop a dilated cardiomyopathy, atrial/ventricular arrhythmias (VA) and heart failure [2]. Animals were maintained on a 12hr light/dark cycle (7AM on - 7PM off). Radio-telemetry monitors (DSI) were implanted and a 24-hour, ambulatory, single-lead ECG was recorded ≥ 5 days later at 400 Hz sampling rate. VA were grouped into 3hr intervals and normalized by total ectopy. ECG analysis was performed using custom software.

Analysis of circadian gene expression

Cardiac tissues were recovered from ~12-month old, wild-type (WT) and TG (female) mice at ~8AM (AM group) and 8PM (PM group). This age/gender was selected, because female TG mice of this age develop heart failure [2]. Hearts were dissected into regions, including left-ventricular (LV) apex and base (without atria), and flash frozen. Isolated total RNA was then used in Real-time PCR (Sybr green) quantitation of BMAL1, per2, and GAPDH expression. Results were normalized to that of GAPDH and relative quantitation performed by the comparative Ct method ($2^{-\Delta\Delta Ct}$) [3].

Circadian patterns to ventricular arrhythmias in humans in ESVEM trial

We examined the 24-hour ECG dynamics that precede initiation of spontaneous, sustained (≥30 sec long) ventricular tachyarrhythmias (VTA) using the largest known database of 24-hour ambulatory recordings collected in the course of an NIH-sponsored, multicenter trial (Electrophysiologic Study Versus Electrocardiographic Monitoring (ESVEM)), which has been described in detail [4]. Patients considered for the ESVEM trial had a history of cardiac arrest, documented ventricular fibrillation, sustained VTA, or syncope. Patients with recent myocardial infarction, the long QT syndrome, hypertrophic cardiomyopathy or arrhythmias due to transient or reversible disorders were excluded. Enrolled patients had at least 10 premature ventricular complexes per hour and inducible sustained VTA. The Holter recordings were obtained at least 5 half lives after the discontinuation of antiarrhythmic drugs; 53 patients were identified for this study by the presence of spontaneous, sustained (≥30 seconds, rate > 100 bpm) VTA.

3. Results

Circadian patterns to ventricular arrhythmias in TG mice

TG mice (n=7) had more frequent PVCs and complex ectopy (couplets, nonsustained ventricular arrhythmias) before and during the activity period: 204 out of 231 complex ectopics occurred between 3PM and 12AM (p=0.01, Figure 1). The amount of ectopy peaked between 3PM and 6PM (p=0.049), prior to the beginning of dark phase, which is associated with increased activity in mice. This upsurge in the incidence of arrhythmias at the beginning of the activity period (dark phase in mice) was strikingly similar to that in humans, who exhibit the greatest incidence of VA in the morning (Figure 2).

Circadian pattern to atrial arrhythmias in TG mice

The incidence of paroxysmal atrial fibrillation in humans increases in the evening and night time [5]. We, therefore, hypothesized that in rodents with nocturnal activity pattern, atrial arrhythmias are more frequent in the morning and day time. Indeed, the frequency of complex atrial ectopy (defined as atrial couplets and longer runs) increased (p=0.07, Friedman ANOVA for repeated measurements) between 1am and 12pm, peaking between 4am and 12pm (Figure 3). The trends were similar when the data were examined in both absolute and normalized units (to control for possible bias toward animals with more frequent arrhythmias, the data were normalized by the total amount of ectopy in each mouse).

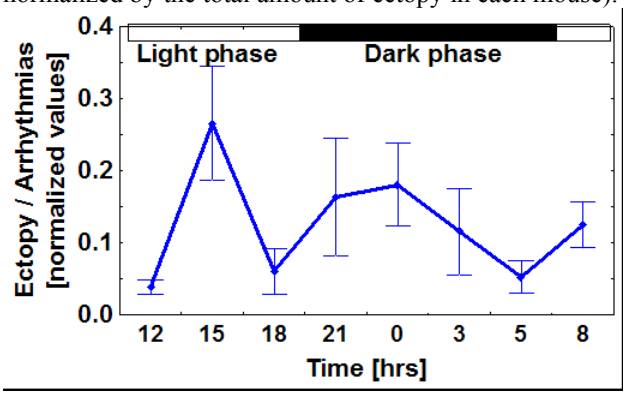

Figure 1. The incidence of ventricular arrhythmias in TG mice peaks before the onset of activity period ("Dark Phase") and remains elevated during that period (p=0.049, Friedman ANOVA, between interval differences). This pattern is similar to that in human patients with reduced left ventricular ejection fraction, which also peaks at the beginning of activity period (in the morning) and remains elevated during day time (Figure 2).

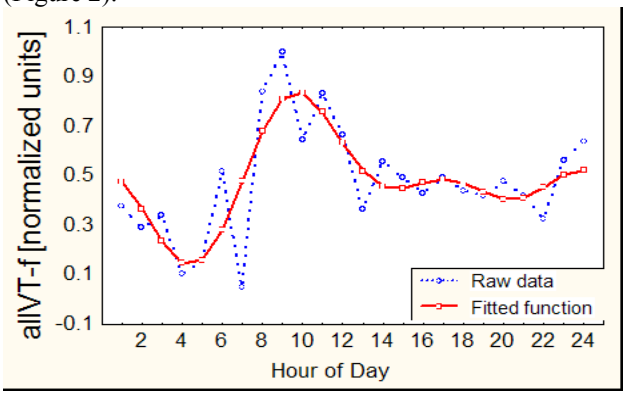

Figure 2. Circadian pattern of occurrence of ventricular tachyarrhythmias (VT), including sustained (≥30 sec) and nonsustained (<30 sec) VT in 53 patients from ESVEM trial. The frequency of VT (allVTf) was normalized by the total number of VT episodes in each patient.

Spatial heterogeneity of Circadian Clock Gene expression in the heart of TG mice

In our previous microarray transcript expression studies performed at a single time point, there were differences between the ventricles of WT and TG mice in the expression of several circadian clock genes [6].

We hypothesized that a spatial heterogeneity of clock gene expression could occur with the development of heart failure and provide a substrate for arrhythmogenesis. Indeed, in both WT and failing TG hearts the RNA levels for two key clock gene transcripts (BMAL1 and per2) showed 12-hour differences

consistent with the circadian pattern of gene expression previously reported for these genes in WT mouse hearts. Furthermore, the level of expression of clock gene transcripts was significantly different in TG versus WT mice in the LV-apex, although LV-base showed a similar level of expression of clock gene transcripts regardless of genotype/heart failure status (Figure 4). These observations support the hypothesis that spatial heterogeneity of clock gene expression occurs in the failing mouse heart.

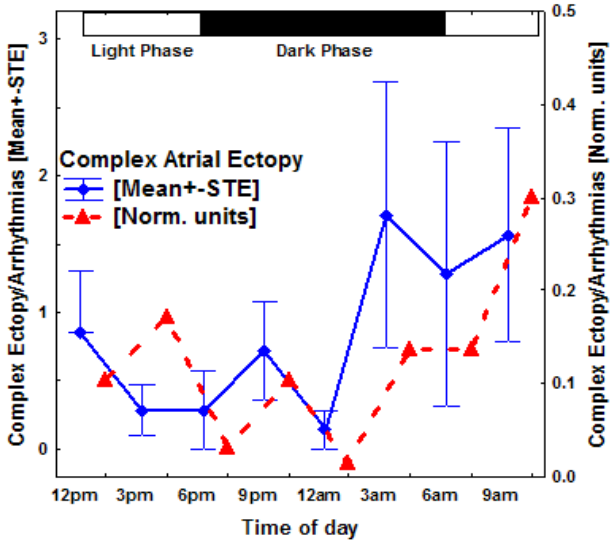

Figure 3. Circadian pattern of complex atrial arrhythmias (defined as atrial couplets or longer runs) in TG mice (N=7). To control for possible bias toward animals with more frequent arrhythmias, the data were also normalized by the total ectopy in each mouse (Norm. units).

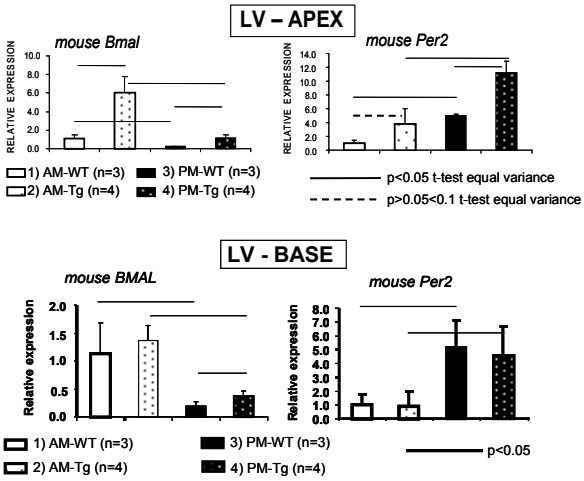

Figure 4. Circadian pattern of BMAL and per2 expression in apical and basal LV tissues of WT and TG mice. N=3-4 mice per genotype/time point combination.

Computational experiments of intracellular circadian clock gene expression in TG and WT mice in the modified Leloup-Goldbeter (LG) model [6]

The findings described in Section 3.3 suggest an altered pattern of circadian clock gene (CCG) expression in failing hearts of TG mice compared to WT mice. This CCG alteration could be linked to the circadian pattern of occurrence of some pathophysiologic processes, in particular, the initiation of arrhythmias. Since the CCG-network is complex and includes multiple molecular interactions, to gain initial insight into the mechanisms and effects of the observed CCG alterations in TG mice, we performed computational experiments as follows.

For simplicity, the simulations were performed using the 16-equation version of LG model, because both the 16 and the 19-equation versions have been shown to produce qualitatively similar results [6]. Since our previous studies in TG mice have demonstrated a significant increase in the expression of Bmal1 mRNA obtained during day time (between 11 am and 5 pm) and since Bmal1 expression is not directly affected by TNF-α, our initial simulations primarily employed modifications of Bmal1 mRNA and its protein (BMAL1). Since TNF-α has been shown to reduce the rate of protein synthesis in the heart [7], we reduced the translation rate for BMAL1 protein by 50% to simulate changes in TG mice (Figures 5 and 6, Run-2) compared to the baseline values corresponding to WT mice (Figures 5 and 6, Run-1). We note that Bmal1 mRNA increased in Run-2 compared to Run-1, which is consistent with our experimental data showing a significant increase in Bmal expression in TG mice compared to controls [8]. The levels of Per mRNA and Cry mRNA decreased in Run-2 compared to Run-1. We also note that the reduction of Per mRNA was greater than that for Cry mRNA (37% and 26%, respectively). These preliminary results are not in disagreement with experimental data showing predominant reduction in Per RNA in mice with cardiac hypertrophy [9]. We also observed the emergence of slow, 25-day periodicity in all time series in Run-2 that was absent in Run-1. This 25-day rhythmicity is produced by lack of coherence and gradual phase shift between the CCG cycling and 24-hour cycle (Figure 6).

Summarizing, these initial computational experiments have shown that: i) the pattern of CCG alterations observed in TG mice can be replicated in a 16-equation LG model, ii) the observed increase in Bmal mRNA could be related to a reduced translation rate of the corresponding protein, and iii) the pattern of CCG alterations in TG mice could lead to the emergence of the 2nd-order, longer periodicities modulating circadian processes. Further computational and molecular-biology experiments are needed to examine the impact of these

slower periodicities on the arrhythmogenesis.

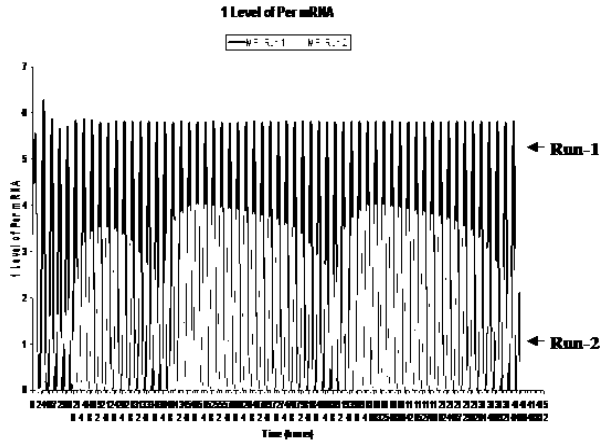

Figure 5. Computational experiments in LG model; level of Per mRNA. Run-1: Baseline, "WT mouse"; (uniform, 24-hour oscillations); Run-2: "TG mouse"; reduced BMAL1 translation rate (lower-amplitude oscillations with a secondary, 25-day periodicity).

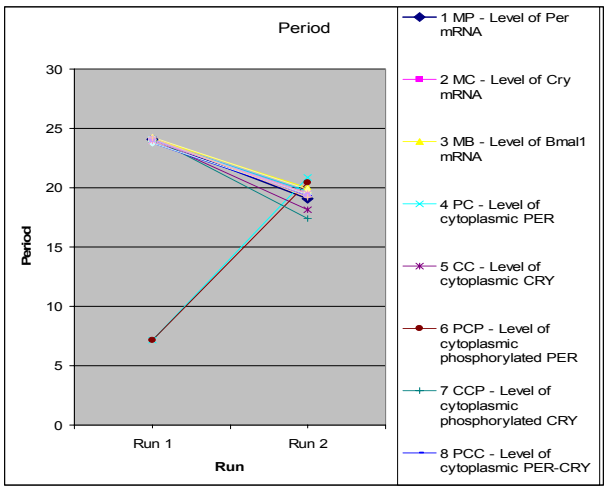

Figure 6. Changes in the period lengths of the circadian network in computational experiments. Run-1: baseline ("WT mice"), Run-2: reduced protein translation rate of BMAL1 ("TG mice").

4. Conclusions

Genetic mouse models of heart failure develop arrhythmias with circadian variation. Similar to humans, ventricular arrhythmias are more frequent shortly before or during the interval of increased activity (human, diurnal; rodents, nocturnal), whereas atrial arrhythmias surge during resting stage.

A murine model of heart failure also showed spatial heterogeneity in the expression of key circadian clock gene transcripts. This might be plausibly translated into the proarrhythmic heterogeneity of cardiac depolarization and/or repolarization.

Since the CCG-networks and their cellular targets involve a number of multi-molecular interactions, computational modeling can be useful for tracking the effects of CCG alterations (observed in TG mice) and pinpointing the links between CCG dynamics and the circadian pattern of arrhythmias. An understanding of these multi-molecular interactions might open a window of opportunity for the development of more effective approaches to arrhythmia prediction and prevention.

Acknowledgements

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References

[1] Hastings MH, Reddy AB, Maywood ES. A clockwork web: Circadian timing in brain and periphery, in health and disease. Nat. Rev. Neurosci. 2003;4: 649-661.

[2] Kadokami T et al. Sex-related survival differences in murine cardiomyopathy are associated with differences in TNF-receptor expression. J Clin Invest. 2000;106:589-97.

[3] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 2001, 25: 402-408.

[4] Shusterman V et al., Autonomic nervous system activity and the spontaneous initiation of ventricular tachycardia. J Am Coll Cardiol 1998; 32:1891-1899.

[5] Shusterman V et al., Patterns of Initiation of Paroxysmal Atrial Fibrillation. Circulation 2002; 106: 458 (Abstract).

[6] Tang ZH et al., Gene expression profiling during the transition to failure in TNF-α over-expressing mice demonstrates the development of autoimmune myocarditis. J Mol Cell Card. 2004;36:515-530.

[7] Lang CH et al., TNF-α impairs heart and skeletal muscle protein synthesis by altering translation initiation Am J Physiol Endocrinol Metab 2002; 282:336-347.

[8] Leloup JC, Goldbeter A. Toward a detailed computational model for the mammalian circadian clock. Proc Natl Acad Sci U S A. 2003;100(12):7051-6.

[9] Young ME, Razeghi P, Taegtmeyer H. Clock genes in the heart: Characterization and attenuation with hypertrophy. Circ. Res. 2001;88;1142-1150.

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