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Cardiac Arrhythmogenesis and Temperature

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***Abstract*—** Fast processes in cardiac electrophysiology are often studied at temperatures lower than physiological. Extrapolation of values is based on widely accepted *Q10* (Arrhenius) model of temperature dependence (ratio of kinetic properties for a 10°C change in temperature). In this study, we set out to quantify the temperature dependence of essential parameters that define spatiotemporal behavior of cardiac excitation. Additionally, we examined temperature's effects on restitution dynamics. We employed fast fluorescence imaging with voltage- and calcium-sensitive dyes in neonatal rat cardiomyocyte sheets. Conduction velocity (CV), calcium transient duration (CTD), action potential duration (APD) and wavelength (W=CV**duration*) change as functions of temperature were quantified. Using 24°C as a reference point, we found a strong temperature-driven increase of CV (*Q10*=2.3) with smaller CTD and APD changes (*Q10*=1.33, 1.24, respectively). The spatial equivalents of voltage and calcium duration, wavelength, were slightly less sensitive to temperature with *Q10* = 2.05 and 1.78, respectively, due to the opposing influences of decreasing duration with increased velocity. More importantly, we found that *Q10* varies as a function of diastolic interval. Our results indicate the importance of examining temperature sensitivity across several frequencies. Armed with our results, experimentalists and modelers alike have a tool for reconciling different environmental conditions. In a broader sense, these data help better understand thermal influences on arrhythmia development or suppression such as during hibernation or cardiac surgery.

I. Introduction

REACTION-DIFFUSION processes such as propagation of excitation waves in cardiac tissue are temperature sensitive. The reaction term incorporates ion channel kinetic properties for excitable membranes while the diffusional component describes passive movement of ions down the electrochemical gradient. In cardiac electrophysiology, the *Q10* is used to describe temperature dependency of ion channel kinetics or, more generally, response of excitable membranes. The *Q10* term was originally derived from the Arrhenius equation for general temperature dependence of rate constants in chemical reactions.

In a classical chemical reaction ($rate = k[A]^x[B]^y \ldots$), the rate constant, k, is the temperature-dependent parameter. This exponential dependence is given by the Arrhenius equation (1889) (4):

$$k = k_o e^{-\frac{E_a}{RT}} \quad [1]$$

describing an exponential decay of some initial rate k_0 with temperature T for a given activation energy E_a and the universal gas constant R. Using the ratio of two rate constants, k_1 and k_2 at two known temperatures, T and T_{ref}, one computes the *Q10* and can infer the apparent activation energy without knowledge of k_0:

$$Q10 = \left(\frac{k_2}{k_1}\right) = e^{\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)}, \text{ for } T - T_{ref} = 10. \quad [2]$$

The value of some measured parameter P_T at a temperature T would be expected to be its value, P_{ref}, at the reference temperature, T_{ref}, scaled by the *Q10* for that parameter:

$$P_T = P_{ref(24°C)} * Q_{10}^{\frac{T-297}{10}} \quad [3]$$

where T_{ref} was chosen to be 24°C (297K). While the Arrhenius model accounts for reaction kinetics, the diffusion of ions is directly proportional to the absolute temperature, following instead Einstein's equation (1905)(3) for the diffusion coefficient.

Temperature dependence in excitable tissue is expected to reflect a combination of reaction (kinetics of depolarizing and repolarizing currents and gap junction proteins) and diffusion (passive ion movement) components. For the small range of temperatures (12°C) examined here, we assumed that *Q10* remained constant over this interval and that the reaction kinetics overwhelm diffusion.

The reaction kinetics are also known to be influenced by the pacing rate or the duration of diastole prior to the excitation, the diastolic interval (DI). Thus, we sought to answer how the three major parameters CV, APD, and CTD vary across both temperature and frequency. Specifically, we were interested in how the restitution slope changed as temperature varied and whether the maximum stable pacing rate was affected by temperature. Lastly, we checked to see if calcium or voltage dynamics lead to the instability.

II. Methods and Material

1) Dynamic functional measurements

Neonatal rat cardiac myocytes were isolated and plated as previously described (2) onto 0.8cm x 2 cm elastic scaffolds.

For experimentation, the cells were washed and equilibrated at room temperature in Tyrode's solution (at

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1.33mM extracellular Ca^{2+}) and co-stained for transmembrane voltage (V_m) and intracellular calcium ($[Ca^{2+}]_i$) with di-8-ANEPPS and Fura-2 AM (both from Molecular Probes, Eugene, OR), respectively. Stained myocytes were then transferred to an experimental chamber perfused with fresh Tyrode's solution at three controlled temperatures, 24°C (T_L), 30°C (T_M), and 36°C (T_H). The order of temperatures tested was varied randomly. Upon completion of recording at one temperature, the cells were equilibrated at a new temperature for 5-10min before repeating the pacing protocol described next. Cells were stimulated with a Pt line electrode at one end of the scaffold, ensuring uniform excitation of cells. Cells were paced at several frequencies starting at 1Hz, gradually increasing frequency, following a dynamic restitution protocol (5). Fluorescence signals were recorded with a photomultiplier tube (PMT) at a fixed distance (1.2-1.6cm) away from the stimulating electrode at a sampling rate of 1000Hz. Steady-state was achieved by pacing for at least one minute. At each pacing frequency, fifteen to twenty transients in first the voltage, then the calcium domain were collected. Cells were paced until they failed to follow 1:1 (occurrence of alternans or blocks in the response), defined here as the breakpoint frequency (f_{break}).

2) Data processing and analysis

Collected fluorescence signals were analyzed with a custom-made Matlab (Mathworks, Natick, MA) program. All frequencies that did not follow 1:1 were excluded from data analysis. CV was estimated from the straight line distance between the stimulating electrode and the lag time between stimulation pulse and acquired response. Aside from CV, transient parameters of interest were action potential duration at 80% repolarization (APD) and calcium transient duration at 80% return (CTD). These parameters were normalized with respect to T_L and thereafter used to calculate wavelength for CTD (W_{CTD}=CVxCTD) and APD (W_{APD}=CVxAPD). In order to calculate *Q10*, linear regression of the logarithm-transformed curves were used with change in temperature as an independent parameter:

$$\ln(P) = \ln(P_o) + \frac{\ln(Q10)}{10}\Delta T \qquad [4]$$

$$Q10 = e^{\frac{10}{\Delta T}[\ln(P)-\ln(P_o)]} \qquad [5]$$

To enable comparison of temperature sensitivity between CV which increases with temperature and durations which decreased with increasing temperature, we report the *Q10*'s for duration as 1/*Q10*.

The restitution behavior was characterized by fitting curves for DI vs. CTD and DI vs. APD using a power fit:

$$y = ax^b + c \qquad [6]$$

with y, representing DI and *x,* duration, and dx/dy or the rate of change of duration with respect to DI was used to locate critical slopes of 1 (6). The dependency of the breakpoint frequency (f_{break}) across temperature was examined to determine which parameter, calcium or voltage, failed 1:1 first.

III. Results

Since a few samples were not usable after the first or second temperature, the reported results have the following *n*: T_L =12; T_M =14; and T_H =12 samples. A typical sample is shown in **Figure 1** demonstrating both the expected restitution behavior at a particular temperature and the direction of change for each parameter (CV increasing while CTD and APD decreasing for increasing temperature).

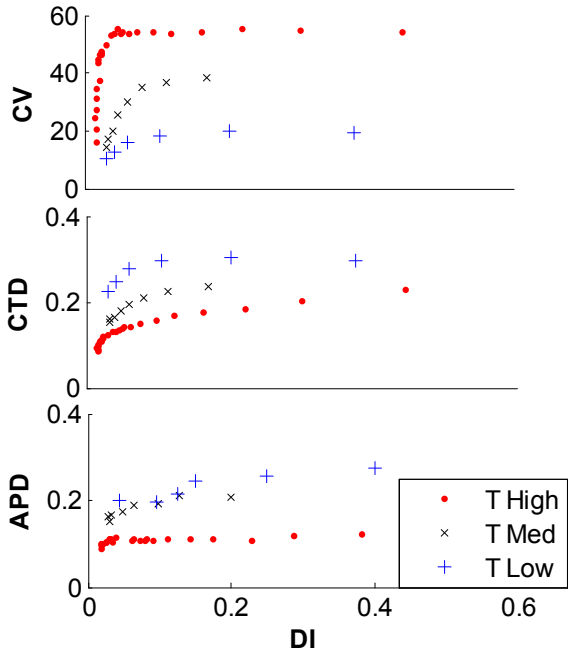

Figure 1: Representative data of temperature response across the frequency range for CV, CTD and APD.

Using a linear regression to fit the normalized average data, and the *Q10* calculation equation [5], we obtained *Q10* values at a particular frequency, 2Hz, displayed in **Table 1**.

Parameter	Calculated *Q10*	R^2 (linear regression)
CV	2.3	0.97
CTD	1.33	0.97
APD	1.24	0.94
W_{CTD}	2.05	1.00
W_{APD}	1.78	1.00
f_{break}	1.64	0.99

Table 1: Calculated *Q10* values via using the *Q10* equation (5) and a linear regression at 2Hz pacing rate.

The number of samples which achieved critical slopes greater than 1 was tallied at each temperature, and the results

shown in **Figure 2**. For T_M and T_H, but not T_L, calcium restitution exceeded 1 more often than voltage. In addition, increasing temperature increased the likelihood of calcium restitution slope exceeding 1 while had little effect on voltage restitution slope.

Next, to see whether these restitution dynamics had any effect on the stability of the samples, we plotted the breakpoint frequency across temperatures and noticed a strong influence as depicted in **Figure 3**. We estimated *Q10* for the relationship of breakpoint frequency (f_{break}) to temperature as indicated in **Table** 1.

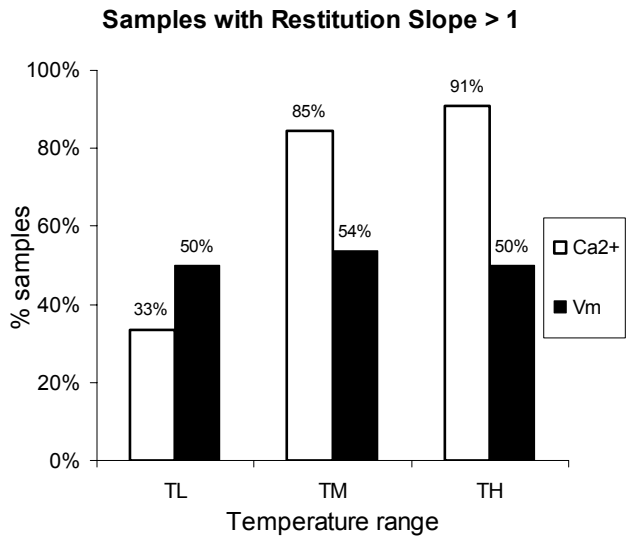

Figure 2: Percentage of samples across temperature exceeding critical slope of 1

Finally, to assess whether the breakpoint was driven by calcium or voltage – defined here as the parameter which failed 1:1 capture first – we enumerated the failure mechanism for each sample across temperature, summarized in **Figure 4**.

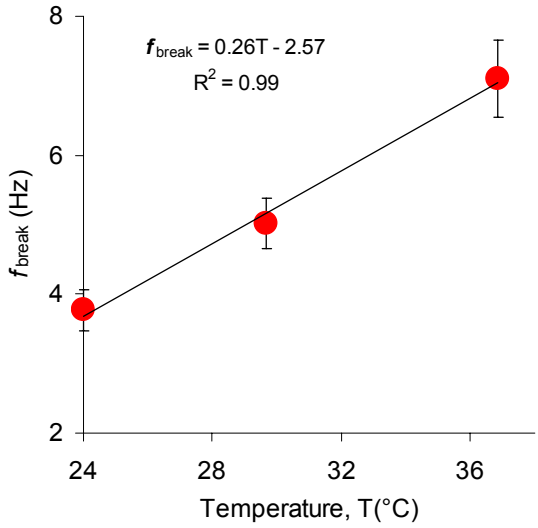

Figure 3: Effect of temperature on breakpoint frequency. Mean values with standard errors are displayed across temperature.

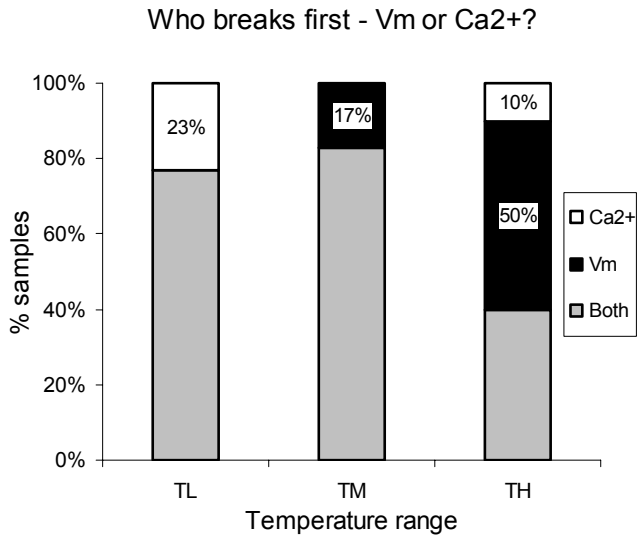

Figure 4: Voltage-Calcium coupling and instability occurrence.

III. Discussion

The *Q10* estimates the temperature sensitivity of each parameter, and we found CV to be the most sensitive followed by wavelength and then duration. While *Q10* estimates have been done before, little attention has been paid to the pacing rate at which the *Q10* measurements were made. Since the kinetics is known to be affected by the DI, we used the percentage of samples exceeding a critical slope of 1 in the restitution plot as an indicator of how frequency and temperature are related. Surprisingly, we found calcium and voltage restitution behavior differed at varying temperatures. Voltage was only slightly affected with a peak at T_M whereas calcium had a clear temperature response with increasing temperature resulting in increased likelihood of instabilities (estimated as exceeding critical slope of 1). The switch in the predominance of calcium initiating instability at T_L to voltage at T_M is surprising given that CTD was less likely than APD to have restitution slopes greater than 1 at T_L but reversed at T_M; hinting that mechanisms other than restitution slope may be responsible. Increasing temperature, in general, made it more likely for voltage to be responsible for breakpoint which coupled with the relative insensitivity of restitution slope to temperature again suggests mechanisms other than critical restitution slope may be underlying the instability.

Our data shows that at higher temperatures, faster kinetics allows the system to handle higher frequency, i.e. higher breakpoint frequencies. Examining a range of temperatures reveals different contribution to instability by Vm and Ca^{2+}. More specifically, Vm may dominate instability occurrence at higher temperatures, while Ca^{2+} may play a more prominent role at destabilizing the system at lower temperatures.

IV. Conclusion

Understanding temperature response can elucidate the fundamental mechanisms of cardiac dynamics. This research will help facilitate collaboration of optical mapping of cardiac excitation under variable experimental conditions. As usual, we found that the *Q10* concept holds relatively well for each of the parameters studied, but that it lacked the capability to describe changes brought about by pacing rate thus limiting the utility of reporting a single *Q10* value. More useful would be a measure similar to *Q10* that describes how *Q10* varies as pacing rate is changed.

We also have evidence that exceeding a critical slope of 1 in the restitution relationship is not necessarily an indicator of instability as have been discussed by others previously (1). Perhaps, like the *Q10* single value model, additional factors need to be considered before restitution slope becomes diagnostic.

Ultimately, these data can help predict thermal influences on arrhythmia development or suppression. Situations where temperature might play a role in arrhythmias include cardiac bypass surgeries where cardioplegia is effected by cold potassium solutions, and in patients exposed to cold temperatures for long durations resulting in hypothermia, a known risk factor for deadly arrhythmias. Better comprehension of the mechanisms underlying temperature-driven instability will aid in the design of therapeutic measures to suppress arrhythmias in temperature-challenged conditions.

Acknowledgment

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