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Variability of action potential duration in pharmacologically induced Long QT Syndrome Type 1

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Abstract—**Long QT Syndrome (LQTS) is a congenital disorder associated with life-threatening arrhythmias. LQT1, a type of LQTS affecting the slow delayed rectifier potassium current, shows a higher incidence of arrhythmia associated with sympathetic stimulation than other types of LQTS. LQT1 patients show increased variability of repolarization with epinephrine infusion, as measured from the 12-lead ECG. We investigate the variability of repolarization measured as action potential duration (APD) in the rabbit left ventricle: how APD variability is affected by pacing rate, transmural location, LQT1 induced by chromanol 293b, and epinephrine infusion. Chromanol preferentially changes APD variability in the midwall. Infusing epinephrine returns the variability to near-control levels. These results differ substantially from clinical studies and show the need for further study.**

I. Introduction

LONG QT syndrome, so called because it usually manifests as prolonged QT intervals on ECG recordings, impairs cardiac repolarization and can lead to dangerous arrhythmias that can cause sudden death. LQTS may be congenital (genetic) or acquired (drug-induced). Congenital LQTS has several forms. The most common forms are LQT1, LQT2, and LQT3, which consist of genetic mutations affecting the slow delayed rectifier potassium current (I_{Ks}), the rapid delayed rectifier potassium current (I_{Kr}), and the inward sodium current (I_{Na}), respectively. Subclinical LQTS, in which mutations are present but show little to no effect under normal conditions, may predispose patients to drug-induced arrhythmia. Some drugs may also induce LQTS conditions in patients without congenital LQTS. A method to identify the proarrhythmic substrate, whether congenital or acquired, is needed.

One method of detecting proarrhythmic substrate in LQTS patients is adrenergic stimulation. Specifically, infusion of epinephrine was observed to increase beat-to-beat variability of the QT interval, in LQT1 patients, but not in normal patients [1]. Beat-to-beat variability of the QT interval has been shown to correlate with increased risk of arrhythmias [2].

In vivo studies of dogs, rabbits, and guinea pigs, which used epinephrine infusion combined with I_{Kr} and I_{Ks} blocking drugs, disagree on whether epinephrine increases beat-to-beat variability of repolarization. However, these studies measured repolarization from ECG or epicardial monophasic action potential recordings [3-6]. Transmural differences in repolarization contribute to arrhythmogenic substrate [7]; therefore transmural differences in repolarization variability are of interest.

This study uses the isolated rabbit left ventricle preparation to record action potentials (APs) and measure action potential durations (APDs) across the heart wall. Thus, we can directly measure transmural changes in beat-to-beat variability of repolarization. Using chromanol 293b to pharmacologically induce LQT1 [8], we can assess the changes in APD variability with epinephrine infusion.

II. Methods

A. Animal preparation

Five adolescent rabbits (7 weeks) were anesthetized with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg), followed by intravenous heparin (800 U/kg) and sodium thiopental (20 mg/kg). Hearts were rapidly excised, weighed, and submerged in cold, oxygenated high-potassium Tyrode solution. The aorta was quickly cannulated and perfused, retrograde, with the same solution. The left ventricular free wall was isolated and the atrioventricular node cauterized. The tissue was then submerged in a heated bath and perfused with warm, normal Tyrode solution, and allowed to equilibrate for 90 minutes. The excitation-contraction uncoupler blebbistatin was added to the perfusate at 5-10 μM concentration and the perfusate was recirculated [9, 10].

B. Optical recording of APs, ECG recording, and signal processing

We used the novel fiber-optic mapping system developed by our group for optical AP recording [10, 11]. Briefly, hearts were stained with the voltage-sensitive dye di-4-ANEPPS (5-10 μM in recirculating perfusate). Excitation light (5 mW green laser) was delivered, and fluorescence was collected, with small-diameter optical fibers (105 μm core, numerical aperture = 0.22), which were arranged across the ventricular wall at four recording locations: subepicardium, upper midwall, lower midwall, and subendocardium. Fluorescence was separated from excitation light using a dichroic mirror, long-pass filtered at 590 nm, and detected with a photodiode. Tissue ECG was recorded with two silver-silver chloride electrodes placed in the bath, with positive electrode and negative electrode placed near the epicardial and endocardial surfaces,

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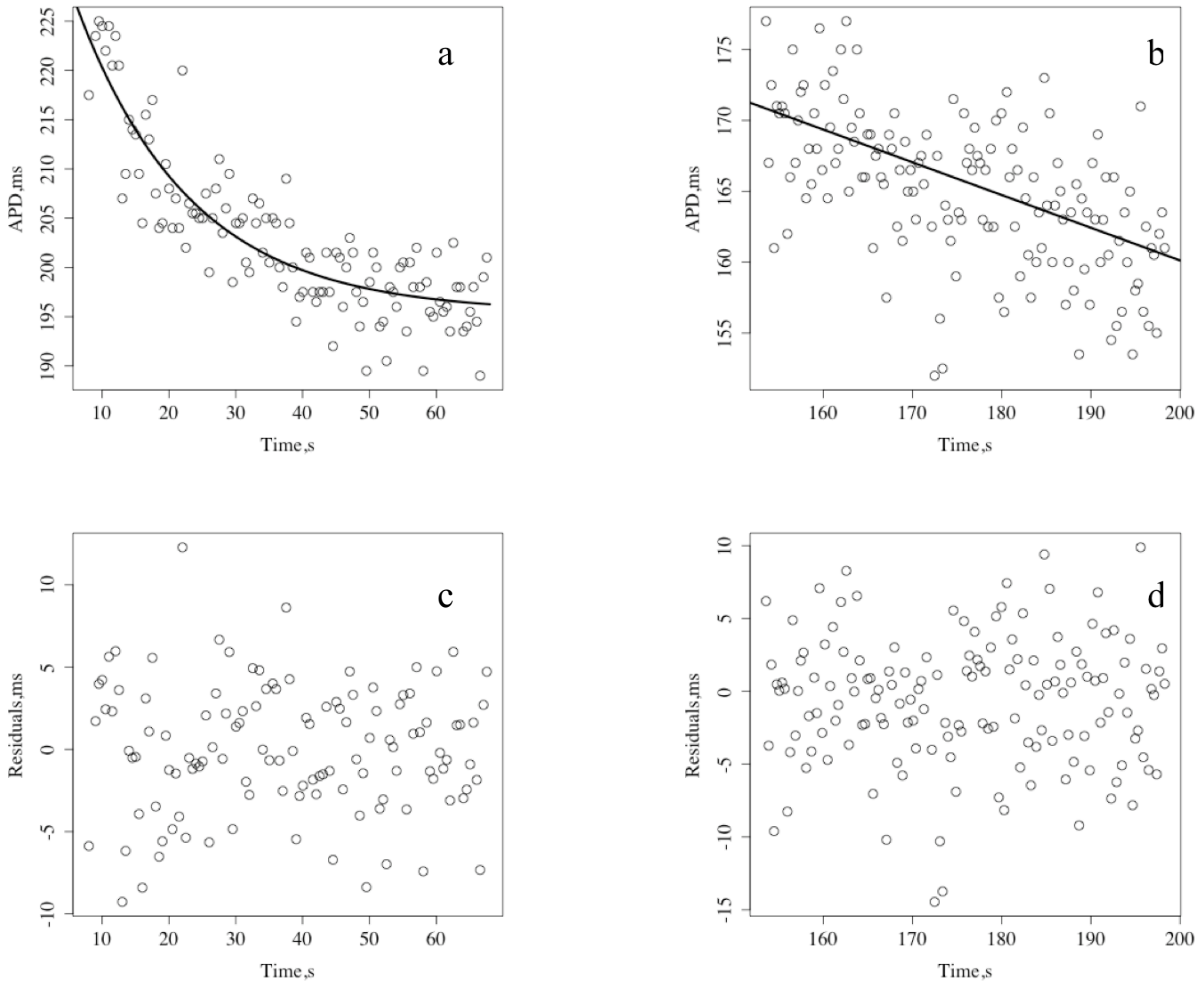

Fig 1. Two transients and their residuals. (a) Transient recorded from the subepicardium under chromanol 293b (30 μM) at BCL 500. It was fit to an exponential function, shown. The residuals are shown in (c); their variance was computed and analyzed. (b) Transient recorded from the lower midwall under chromanol 293b 30 μM plus epinephrine (0.5 μM) at BCL 300. It was fit to a linear function. The residuals are shown in (d); their variance was computed and analyzed.

respectively. Optical and ECG signals were amplified, low-pass filtered (500 Hz), sampled at 2 kHz, and digitally recorded on a hard drive.

C. Pacing protocol

We used a downsweep pacing protocol developed by our group [9, 12]. Briefly, the protocol begins with constant pacing at an initial basic cycle length (BCL), BCL0 (typically 1000 ms). Data collection begins at steady-state during BCL0 pacing. Pacing is then stepped to a shorter BCL, BCL1, for 45 seconds. Then a single S2 stimulus is delivered at a BCL of BCL1 + Δ, where Δ is typically 20-50 ms. Pacing at BCL1 resumes for 5 beats, and then a single S2 stimulus at a BCL of BCL1 - Δ is delivered. Pacing at BCL1 resumes for 20 beats. The BCL is decremented again to BCL2, and the cycle repeats. For this study, BCLs were 500, 400, 300, 250, 200, 180, 160, 140. The complete set of decrements is considered a complete downsweep. Tissue was paced from the epicardial surface at twice diastolic threshold measured under control condition (see Section D).

D. Drug conditions

For each animal, recordings were made under four drug conditions. One downsweep was performed and recorded under control condition (recirculating perfusate as described in Section A). Then epinephrine (0.5 μM) was added to the perfusate, allowed to equilibrate for 15 minutes, and a second downsweep was recorded. Epinephrine was washed out with clean, non-recirculating Tyrode solution for at least 30 minutes. Then the heart was perfused with a solution of 30 μM chromanol 293b and 5-10 μM blebbistatin in 250 mL Tyrode solution, recirculating. After a 30 minute equilibration period, a third downsweep was recorded. 0.5 μM epinephrine was added to the perfusate (so that the perfusate contained both chromanol and epinephrine), allowed to equilibrate 15 minutes, and a fourth and final downsweep was recorded. Tissue was re-stained with di-4-ANEPPS before each recording as necessary.

E. Data analysis

APDs were extracted using custom-written software in MATLAB (MathWorks, Natick, MA). Baseline oscillations in fluorescence were subtracted, APs identified, and APDs computed at 70% of full repolarization. For APD variance analysis, we considered only the APDs during each 45-second constant-BCL pacing segment, referred to as the *transient*. APD variance was computed as follows: For each

Table 1. Log of variance of APD residuals by drug condition and transmural location.

Drug	Subepicardium	Upper midwall	Lower midwall	Subendocardium
Control	2.33 ± 0.58	2.03 ± 0.27	3.31 ± 0.68	2.64 ± 0.61
Epinephrine 0.5 μM	2.95 ± 0.61	2.82 ± 0.23*	3.66 ± 0.65†	3.52 ± 0.69
Chromanol 30 μM	3.13 ± 0.52	3.00 ± 0.66*	2.85 ± 0.44	2.77 ± 0.73
Chromanol 30 μM + Epinephrine 0.5 μM	2.79 ± 0.84	2.21 ± 0.79	3.63 ± 0.57†	2.56 ± 0.31

Values are reported as mean ± SD. * represents p<0.05 vs. control; † represents p<0.05 vs. chromanol.

BCL under each drug condition at each transmural location, the transient (APD vs. time *t)* was fit to a decaying exponential $APD(t) = a + be^{-t/\tau}$. If the standard error of τ was greater than twice the estimated value of τ itself, the transient was instead fit to a straight line. The fit was subtracted and the variance of the residuals computed (see Fig. 1). If pacing did not yield a 1:1 response (an AP for every pacing pulse) under any combination of conditions, the variance of that transient was omitted from analysis.

The foregoing analysis yielded a data set of 505 APD variances, one for each combination of 5 animals, 4 drug conditions, 8 BCLs, and 4 transmural recording locations (except for omitted data). The APD variances over all animals under matching drug, location, and BCL conditions were averaged. This analysis yielded a data set of 120 APD variances, one for each combination of 4 drug conditions, 8 BCLs, and 4 recording locations (except for omitted data).

F. Statistics

A linear model was fit to the APD variance data using the statistical program R (R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org). The response variable was the logarithm of APD variance. BCL was a continuous variable. Factors were drug, recording location, and their interaction. Significance threshold was considered to be $p < 0.05$.

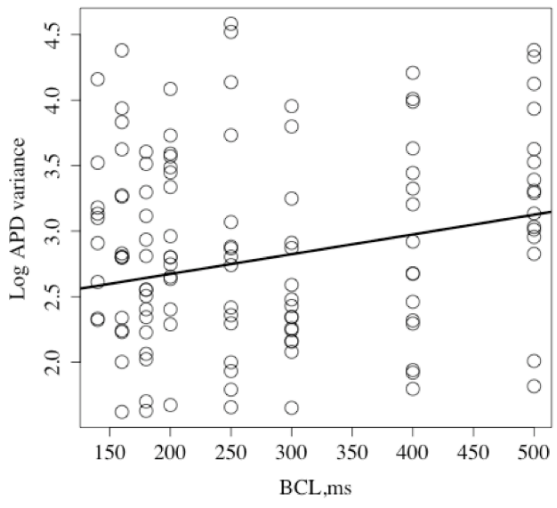

Fig 2. Points: log of APD variance vs. BCL for all locations and drug conditions. Line: shows the increase of log of APD variance with BCL (slope=1×10^{-3}).

III. Results

A. Effect of BCL on variance

Logarithm of APD variance showed a small, but significant, increase with increasing BCL (p=0.03) (see Fig. 2). There was no significant interaction between drug condition and BCL.

B. Effect of drug on variance

When transmural locations were pooled, logarithm of APD variance increased significantly relative to control conditions (mean±SD: 1.77±0.31) with 0.5 μM epinephrine (2.56±0.31, p=0.005) and with 30 μM chromanol 293b (2.72±0.26, p=0.02), although logarithm of variance did not differ significantly between epinephrine and chromanol conditions (p=0.59). Under chromanol+epinephrine conditions, logarithm of APD variance was not significantly different from control conditions (1.94±0.32, p=0.29).

C. Transmural heterogeneity of APD variance

As shown in Table 1, while APD variance did not show significant differences over transmural location alone, the interaction of drug condition with transmural location was significant (p=0.01). Increase in variability under epinephrine or chromanol was more pronounced in the midwall than in the subepicardial or subendocardial locations.

D. Variance and AP shape

Since prolongation of the AP plateau can be expected to correlate with increased APD variance [13, 14], we compared AP shapes between drug conditions at each transmural location. No consistent differences were observed (data not shown).

IV. Discussion

Our study found that changes in APD variability were associated with changes in pacing rate, pharmacological conditions, and transmural location. Chromanol preferentially changes APD variability in the midwall.

Infusing epinephrine returns the variability to near-control levels.

The differences in drug effects between the two midwall locations may be caused by a combination of the differential transmural effects of adrenergic stimulation [7], and spatial averaging in the optical signal [15] . Note that midwall cells are not necessarily M cells; our study did not observe the longer APD characteristic of M cells [7].

Contrary to the findings of the clinical study in [1], chromanol 293b-induced LQT1 in the rabbit left ventricle does show increased APD variability compared to control (non-LQT) conditions. Furthermore, epinephrine infusion to the rabbit left ventricle chromanol preparation seems to return APD variability to near-control levels rather than increasing it further. The study in [1] was based on ECGs recorded during sinus rhythm, without pacing. Our paced, isolated left ventricle preparation shows a different APD variability response.

V. Conclusion

The results of our study suggest that the effect of epinephrine in APD variability measured in paced, *in vitro* preparations is different from its effect measured clinically using the ECG. It would be of interest to compare these APD variability results with beat-to-beat QT variability measured from the ECGs recorded during the same study. Further studies are needed to elucidate the connection between APD variability, adrenergic stimulation, and proarrhythmia under LQTS conditions.

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