Measurement and interpretation of electrocardiographic QT intervals in murine hearts

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Alterations in ECG QT intervals correlate with the risk of potentially fatal arrhythmias, for which transgenic murine hearts are becoming increasingly useful experimental models. However, QT intervals are poorly defined in murine ECGs. As a consequence, several different techniques have been used to measure murine QT intervals. The present work develops a consistent measure of murine QT interval that correlates with changes in the duration of ventricular myocyte APs. Volume-conducted ECGs were compared with simultaneously-recorded APs obtained using floating intracellular microelectrodes in Langendorff-perfused mouse hearts. QT intervals were measured from the onset of the QRS complex. The interval, Q-APR$_{90}$, measured to the time at 90% AP recovery, was compared with two measures of QT interval. QT1 was measured to the recovery of the ECG trace to the isoelectric baseline for entirely positive T-waves, or to the trough of any negative T-wave undershoot. QT2, extensively used in previous studies, was measured to the return of any ECG trough to the isoelectric baseline. QT1, but not QT2, closely correlated with changes in Q-APR$_{90}$. These findings were confirmed over a range of pacing rates, in low [K$^+$] solutions, and in Scn5a$^{+/\Delta KPQ}$ hearts used to model human long QT syndrome. Application of this method in whole anaesthetized mice similarly demonstrated a prolonged QTc in Scn5a$^{+/\Delta KPQ}$ hearts. We therefore describe a robust method for the determination of QT and QTc intervals that correlates with the duration of ventricular myocyte action potentials in murine hearts.

Key words: action potential duration, ECG, QT interval, mouse heart
The ECG QT interval reflects the duration of ventricular depolarization and repolarization (1, 13). It is important for the diagnosis of inherited and iatrogenic QT disorders associated with increased risks of polymorphic ventricular tachycardia or torsades de pointes that may present as long or short QT syndromes. Transgenic mouse hearts have proven increasingly useful in modelling human cardiac arrhythmic disease. However, mice differ from humans in aspects of cardiac electrophysiology that have implications for ECG interpretation. Murine hearts show shorter electrocardiographic RR intervals reflecting higher heart rates (600 bpm).

Murine QRST complexes reflect considerably shorter AP waveforms lacking plateau phases resulting in indistinct ST segments and T waves (12). Nevertheless the T-wave is often assumed to end at its return to the isoelectric baseline (5, 9). However, where the T wave in some mouse ECG traces shows an apparent negative undershoot in addition to its positive component this would substantially increase estimates of the QT interval, or preclude determination of QT intervals at high heart rates when the P wave often becomes superimposed upon the terminal phase of the previous negative undershoot (12).

Previous murine studies had adopted different empirical QT estimation methods including determining: (A) the time taken to reach 95% recovery for the ECG deflection to return to baseline (10); (B) the time to the end of the T-wave, itself defined as the point at which the T wave timecourse deviates from the tangent to the steepest slope of timecourse of T-wave recovery (9); (C) the time at which the QT segment returns to baseline (2, 3, 6); and (D) the point of convergence of T waves and their first derivative on a signal-averaged ECG recording (14). These various approaches met with variable success, leading to doubts as to whether ECG analysis of this kind could quantify murine action potential parameters (4).

The present paper develops a simple empirical approach for measuring the QT interval in mouse ECGs by comparing ECGs with simultaneous intracellular recordings of AP waveforms in
Langendorff-perfused hearts. It then tests the resulting criteria over a range of pacing rates and under conditions of altered QT interval, in low [K⁺] solutions and in Scn5a+/ΔKPQ hearts modelling long QT syndrome. It then further compares in vitro recordings with QT intervals recorded in intact anaesthetized Scn5a+/ΔKPQ mice.

METHODS

Experimental Animals

Experiments were conducted using WT and Scn5a+/ΔKPQ mice inbred on a 129/Sv genetic background, aged 3-6 months housed in cages at 21±1°C with 12 h light/dark cycles. All procedures were performed in institutional premises approved under the UK Animals (Scientific Procedures) Act (1986), under UK Home Office project licence no. PPL No. 80/1974, approved by a university ethics review board, accordingly also in conformity with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Simultaneous epicardial ventricular action potential and volume-conducted electrocardiographic (ECG) recordings from intact Langendorff-perfused hearts

Mice were killed by cervical dislocation (Schedule 1: UK Animals [Scientific Procedures] Act 1986). Their aortas were cannulated and the heart was perfused at a constant flow rate of 3 ml min⁻¹ (Watson-Marlow Bredel Peristaltic pumps, model 505S, Falmouth, Cornwall, U.K.) with Krebs-Henseleit (KH) solution (mM: NaCl 119, NaHCO₃ 25, KCl 4, KH₂PO₄ 1.2, MgCl₂ 1, CaCl₂ 1.8, glucose 10 and Na-pyruvate 2; pH adjusted to 7.4) bubbled with 95% O₂/5% CO₂ (British Oxygen Company, Manchester, UK) on a Langendorff system. The KH solution was passed through a 5 µm filter (Millipore, Watford, UK) and warmed to 37°C using a water jacket and circulator (Techne model C-85A, Cambridge, UK). Hearts were laid down with their anterior surfaces facing upwards in a home-made warmed bath chamber. Only hearts that regained their pink colour and showed 1:1
atrioventricular conduction with intrinsic activity and after 10-15 min perfusion for stabilization were then subjected to further electrophysiological testing.

A floating microelectrode holder was constructed from a thin, coiled silver wire (0.4 mm in diameter) and connected to a 2 mm connector. A glass micropipette was drawn from borosilicate glass to a very fine tip and filled immediately prior to use with 3M KCl. The pipette was cut above its shoulders and the remaining shank was discarded. The micro-electrode resistances were 15 to 25 MΩ. The chlorided end of the silver wire was inserted into the micropipette, with which impalements were made close to the midpoint between ventricular apex and base, and connected to a to a high input impedance DC microelectrode amplifier system (University of Cambridge, Cambridge, UK). The signals were displayed, digitized and analysed using Spike 2 (Spike 2, Cambridge Electronic Design, Cambridge, UK). Conversion of the analog input to digital signals was performed using a model micro1401 interface (Cambridge Electronic Design, Cambridge, UK) connected to an IBM-compatible computer. Spike II software (Cambridge Electronic Design, Cambridge, UK) was used to record and subsequently analyse electrocardiogram recordings. The entire apparatus was mounted on a vibration isolation platform in a grounded Faraday cage. Action potentials showing straight upstrokes, with amplitudes (APA)>75mV, maximum rates of rise (dV/dt)\(_{\text{max}}\)>85mV/ms, resting potentials (RPs) between -80 to -65mV were used for further analysis.

Volume-conducted ECGs were recorded simultaneously with the action potential recordings. Three needle electrodes were immersed in the superfused bath flanking the isolated heart. Signals were amplified and filtered by a model NL104A amplifier (Neurolog, Digitimer. UK), and a model NL125/126 filter (set to a bandwidth of 10 to 5000 Hz). Conversions of analogue input to digital form used a model 1401+ interface (Cambridge Electronic Design, Cambridge, UK) connected to an IBM-compatible computer. Spike II software (Cambridge Electronic Design, Cambridge, UK) was used to record and subsequently analyse electrocardiogram recordings.

An initial series of experiments studied hearts in sinus rhythm. Further experiments assessed the ECG measured under conditions of regular stimulation at cycle lengths (CL) of 200, 167 and 143 ms, at
which 50 APs were recorded for each CL. This used a bipolar platinum stimulating electrode (1 mm inter-pole spacing) placed against the right atrial epicardium delivering square-wave stimuli (Grass S48 stimulator, Grass-Telefactor, Slough, UK) of 2-ms duration and amplitudes of twice diastolic excitation threshold. The experiments also examined the effect of reducing the extracellular K+ concentrations from normokalaemic (4 mM) to hypokalaemic (3 mM) levels in the K-H perfusate to investigate the effects of conditions that would prolong the QT interval and its measurement.

Measurements from the ECG and intracellular action potential traces

The duration of ventricular electrophysiological activity was determined by three measurements from the ECG and the AP traces. In each case, peaks and recovery times were obtained from direct readouts from successive averages of 5 data points obtained by software cursors successively moved along the time axis. In the ECG traces, the position of the isoelectric baseline was defined as falling between the end of the upright P wave and the beginning of the QRS in the volume-conducted ECGs as on previous occasions (15). The beginning of ventricular electrical activity was defined by the onset of the QRS in the ECG trace where it deviated from this iso-electric baseline. The QT interval was then calculated by two methods: QT1 was measured to the time at which the ECG trace first reached its minimum value; this was the iso-electric baseline when the T wave only showed positive components, or the point of maximum undershoot where records showed a negative undershoot. QT2 was defined as the time at which the T wave reached the isoelectric baseline. Corrected QT intervals (QTc) were then obtained using the formula QTc= QT/(RR/100)\(^{1/2}\) to give Q-APR\(_{90c}\), QTc1, and QTc2 respectively (11). Finally, in the intracellular microelectrode recordings, the time of 90% recovery of the ventricular AP from its peak to the iso-electric baseline was found. For comparison with ECG records, the Q-APR90 was defined as the time intervening between the start of the ECG QRS complex and the time of the APR90.

In vivo ECG recordings from mice under terminal anaesthesia
Independent, in vivo, ECG studies were performed on mice anaesthetized with a dose of 0.10 ml/10 g body weight, of either (A) a solution comprising 1.8 ml of 100 mg/ml ketamine hydrochloride (Ketaset, Fort Dodge, Southampton, UK), 0.35 ml of 23.32 mg/ml xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany) and 2.85 ml of sterile phosphate base solution or (B) 24 mg/ml Avertin (2,2,2-tribromo-ethanol, Sigma, Poole, Dorset, UK), intraperitoneally into the left peritoneal cavity, respectively 15 and 5 min before recording. The anaesthetized mice were placed on a heating pad with continuous monitoring of body temperature for three-lead ECG measurements in lead II over 10 min using subcutaneous needle electrodes and a Powerlab 26T system (AD Instruments, Hastings, UK). Recordings (16 bit, 2 kHz/channel) were analysed using the Chart v6.0 program (AD Instruments, Oxfordshire, UK). Recordings were filtered between 0.5 and 500 Hz.

**Data analysis and statistics**

Data are expressed as means ± SEM. The numbers, n, denote either numbers of whole hearts, or the number of cells. The sets of data were compared using ANOVA with post hoc Tukey’s honestly significant different tests as well as regression analysis (SPSS software). P values less than 0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

Fig. 1 displays typical ECGs from a range of Avertin-anesthetized mice studied under similar conditions. Isoelectric baselines were determined between the end of the P wave and the beginning of the QRS (16). The beginning of ventricular electrical activity was defined by the beginning of the QRS complex. As reported previously in both mouse and rat, the beginnings of the T waves were often indistinct likely reflecting short or absent AP plateau phases (12, 17).

Fig. 1A shows an upright ECG T wave with a clear-cut decay to isoelectric baseline (arrow). In contrast, in Fig. 1B, the positive component of the T wave was followed by a prolonged negative undershoot (arrow). Inclusion of the latter waveform would thus substantially increase estimates of QT interval (40 ms vs. 130 ms respectively). Fig. 1C shows T waves with negative undershoot.
components not clearly separable from the succeeding P waves, which would complicate
determination of the end of the T wave.

Fig 2A compares typical intracellular ventricular APs (a) obtained in WT hearts with simultaneously
recorded volume-conducted ECGs (b). The latter either showed positive-going T waves followed
either by direct decays (i), or by prolonged negative undershoots (ii). Different definitions of QT
interval, as depicted by the cursors, were compared with predictions from AP recordings. Thus, cursor
1 shows Q-APR_{90}, extending to the time at 90% AP recovery in the AP trace. Cursor 2 depicted QT1,
extending to the point at which ECG trace regained the isoelectric baseline in records where the T
wave only showed positive components, or where any prolonged negative undershoots reached their
minima. In this example, QT1 was in agreement with Q-APR_{90} in each case (within ~9.8 ms in 2Aii).
In contrast, QT2, which extended to full recovery to the isoelectric baseline (cursor 3), gave marked
104.7 ms differences from Q-APR_{90} with the waveform depicted in Fig. 2A(ii).

Fig. 2B plots QT1 and QT2 against Q-APR_{90} in each of 24 myocytes from 8 WT hearts, including
means and SEs of the mean where the latter exceeded the sizes of the data points themselves. Each
point shows ECG and intracellular action potential parameters obtained simultaneously and averaged
over the full duration of each successful cell impalement. The regression lines showed that QT1
closely correlated with Q-APR_{90} (QT1 = 0.986 × Q-APR_{90}; regression coefficient r = 0.863). In
contrast QT2 values correlated poorly with Q-APR_{90} (QT2 = 2.15 × Q-APR_{90}; r = 0.618), as
previously demonstrated (4).

Fig. 2C illustrates the closeness or otherwise of Q-APR-90, QTc1 and QTc2 for each of the 8 hearts
that were studied. In each of these individual hearts, Q-APR_{90c} and QTc1 values were statistically
indistinguishable (P>0.05, n= 8). In contrast, QTc2 values were consistently larger than both Q-
APR_{90c} and QTc1 (Fig. 2C). Concordant results emerged from comparisons between Q-APR_{90c} and
QTc1 (P>0.05), but not between these measures and QTc2 (P<0.01 in both cases), over the entire set
of hearts (Fig. 2D). Hence QTc1 correlated well with Q-APR_{90c} over a wide range of values, whereas
QTc2 is larger than Q-APR90c and correlates with it poorly, especially when the T-wave has negative components.

Measurements of Q-APR90, QT1 and QT2 intervals, averaged over 50 beats, were then repeated during pacing at cycle lengths of 200, 167 and 143 ms. Means and standard errors at the three pacing rates were similar for (a) Q-APR90 (41.04 ± 0.09; 41.55 ± 0.05 and 41.02 ± 0.04 ms respectively) and (b) QT1 (42.78 ± 0.17; 42.47 ± 0.19 and 41.66 ± 0.46 ms). In contrast, both means and the standard errors of QT2 markedly varied at the three pacing rates (109.56 ± 0.74; 101.76 ± 0.77 and 73.03 ± 0.58 ms respectively), and significantly differed from Q-APR90 in each case.

Further measurements of Q-APR90, QT1 and QT2 were made before and after acute alterations in action potential durations produced by replacing normokalemic with hypokalemic perfusate, previously shown to produce prolongation of action potential duration as recorded using MAP electrodes (8). Hypokalemia increased Q-APR90 from 40.89 ± 1.08 (4 cells in one heart) to 44.27 ± 0.72 ms respectively (8 cells in one heart). Simultaneously-recorded QT1 values similarly increased, from 41.02 ± 2.49 to 53.08 ± 0.37 ms. In contrast, the corresponding QT2 values reduced from 95.79 ± 3.22 to 89.00 ± 3.89 ms respectively.

QT intervals were then quantified in murine Scn5a+/-ΔKPQ hearts containing the ΔKPQ(1505-1507) gain-of-function Scn5a deletion modelling human long QT syndrome and expected to demonstrate chronic QT interval prolongation (7, 18). Ventricular APs were obtained from 40 cells in three Scn5a+/-ΔKPQ hearts. An example is shown in Fig. 3A(ii) for comparison with a WT recording (Fig.3A(i)). These showed greater AP durations than WT (Q-APR90c values of 41.47 ± 3.37 in Scn5a+/-ΔKPQ vs. 36.33 ± 1.45 in WT, P < 0.05). Simultaneously-recorded volume-conducted ECGs similarly demonstrated increased values of QTc1 (43.58 ± 4.22) and QTc2 (111.3 ± 5.91) relative to WT (35.99 ± 1.33, P < 0.05 and 66.92 ± 6.21, P < 0.01, respectively). As for the WT hearts (Fig. 2), mean QTc1 values in Scn5a+/-ΔKPQ hearts (Fig. 3C) were not significantly different from Q-APR90c.
(43.58 ± 4.22 vs. 41.47±3.37, P>0.05) whereas their mean QTc2 value (111.3±5.91) was significantly larger than both Q-APR90c and QTc1 (P < 0.01 in each case).

Finally (Table 1), ECGs were recorded from intact anaesthetized as opposed to isolated perfused Scn5a+/ΔKPQ and WT mice. These were statistically indistinguishable (P > 0.05) within groups under either Avertin (n = 19 and 26 respectively) or ketamine anaesthesia (n = 10 and 23). However, Scn5a+/ΔKPQ showed consistently greater QTc1 than WT (44.56±2.23 and 45.42±3.00 in Scn5a+/ΔKPQ vs 33.60±1.87 and 30.75±1.50 ms in WT; P<0.05 in both cases) in agreement with findings in isolated perfused hearts.

Thus, the time from the start of ventricular activity to the time of minimum voltage (QT1) is a stable and repeatable measurement of the murine QT interval that correlates with the duration of intracellularly-recorded action potentials.

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DISCLOSURES

None
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    gain-of-function Scn5a+/ΔKPQ hearts suggest an overlap syndrome. Am. J. Physiol. Heart 
Table 1. Summary comparing QTc intervals in anaesthetised mice during ECG recordings.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genotype</th>
<th>n=hearts</th>
<th>QTc (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avertin</td>
<td>WT</td>
<td>26</td>
<td>33.60±1.87*</td>
</tr>
<tr>
<td></td>
<td>Scn5a+ΔKPQ</td>
<td>19</td>
<td>44.56±2.23*</td>
</tr>
<tr>
<td>Ketamine</td>
<td>WT</td>
<td>23</td>
<td>30.75±1.50§</td>
</tr>
<tr>
<td></td>
<td>Scn5a+ΔKPQ</td>
<td>10</td>
<td>45.42±3.00§</td>
</tr>
</tbody>
</table>

Note: Similar symbols differ from each other at P<0.05 for single symbol, P<0.01 for double symbols.
**FIGURE LEGENDS**

**Fig. 1** ECG patterns from anaesthetised mice show marked variability.

In (A), recorded from a WT mouse, the QRS-T complex ends with an upright T wave that then returns to the isoelectric baseline (arrow). In (B), recorded from an Scn5a\(^{+/-}\Delta KPQ\) mouse, the positive part of the T wave is followed by a negative undershoot before returning to the isoelectric baseline (arrow). In (C), recorded in a WT mouse 10 min after administration of isoprenaline (2.0mg/kg i.p.), the T waves show negative undershoot components not fully separable from the succeeding P waves.

**Fig. 2** Relationships between intracellular AP duration and volume ECG QT intervals in WT hearts.

Panels A(i) and (ii) show (a) APs and (b) the corresponding ECG recordings. Cursor 1 indicates the AP at 90% recovery (Q-APR\(_{90}\)). Cursor 2 is placed at the minimum value of a late negative undershoot (QT1). Cursor 3 indicates the point where the undershoot component regains the isoelectric baseline (QT2). (B) plots QT1 and QT2 intervals against Q-APR\(_{90}\) values observed and their respective regression lines. "(C) summarizes Q-APR\(_{90c}\), QTc1 and QTc2 results from 8 individual hearts. Results from hearts 1-8 were obtained in the course of recording from n = 6, 4, 3, 1, 5, 1, 1 and 3 cells respectively, of which ECG records in hearts 2 and 6 showed a kinetic pattern generally similar to that in A(i)(b), while the remainder showed more complex kinetic patterns similar to that in A(ii)(b). (D) summarizes mean (±SEM) Q-APR\(_{90c}\) and QTc1 and QTc2 values.

**Fig. 3** Relationships between intracellular AP duration and volume ECG QT intervals in Scn5a\(^{+/-}\Delta KPQ\) hearts.
(A) compares simultaneous recordings of a typical AP from a single ventricular myocyte (a) with the corresponding ECG trace (b) from WT (i) and Scn5a+/ΔKPQ (ii). (B) compares Q-APR90c, QTc1 and QTc2 in individual Scn5a+/ΔKPQ hearts; (C) summarizes their mean (±SEM) Q-APR90c, QTc1 and QTc2 values.