Development and characterization of a mouse in vitro model of ischaemia-induced ventricular fibrillation

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Received 27 October 2008; revised 6 February 2009; accepted 17 February 2009; online publish-ahead-of-print 19 February 2009

Time for primary review: 21 days

Aims We sought to generate a mouse Langendorff model of ischaemia-induced ventricular fibrillation (VF) that does not depend on triggers such as programmed electrical stimulation.

Methods and results Hearts from male Tuck Ordinary mice were perfused with Krebs solution (modified to contain low-normal K⁺, 3 mmol/L, and high Ca²⁺, 2.4 mmol/L) containing different combinations of catecholamines (epinephrine 313 nmol/L plus norepinephrine 75 nmol/L) and/or angiotensin II (100 pmol/L) designed to mimic the in vivo milieu. VF was absent during 30 min regional ischaemia (and during 10 min reperfusion) in Krebs-perfused hearts. Catecholamines unmasked ischaemia-induced VF (50%; $P<0.05$) and reperfusion-induced VF (50%; $P<0.05$). Co-perfusion with angiotensin II did not facilitate VF. Supraventricular pacing (600 b.p.m.) stabilized pre-ischaemic sinus rhythm and partially mimicked the VF-unmasking effect of catecholamines. Arrhythmia susceptibility was greatest with supraventricular pacing plus catecholamines (57% VF during ischaemia and 71% during reperfusion).

Conclusion For the first time, regional ischaemia-induced VF was consistently evoked in a mouse Langendorff preparation, unmasked by simple periphysiological manipulation of the perfusion conditions. The model is suitable for functional genomic studies.

KEYWORDS
Antiarrhythmic agents; Ischemia; Mouse models; Sudden death; Ventricular arrhythmias

1. Introduction

Approximately 50% of coronary artery disease (CAD)-related deaths are sudden and unexpected,¹ with many due to ventricular fibrillation (VF) triggered by acute myocardial ischaemia.²,³ There is a great challenge to develop safe and effective antiarrhythmic drugs. This requires more detailed understanding of the mechanisms of ischaemia-induced VF, which requires animal models in which VF is elicited by coronary artery ligation.

Transgenic (TG) mice have been used to test hypotheses about arrhythmogenicity.⁴ However, although it is possible to evoke VF in mouse hearts by using CAD-unrelated triggers,⁵–⁷ attempts to obtain reproducible coronary ligation-induced VF in mouse models have failed. Only one study has obtained ischaemia-induced VF⁸ (in just 2/13 wild-type mice), with several other studies reporting no VF at all.⁹–¹⁵ Even reperfusion-induced VF, which is easy to elicit in most species,¹⁶ has been reported in only one in vivo mouse study,¹⁷ with other similar studies showing no VF.¹⁸,¹⁹,²⁰ Consequently, all current mouse models use surrogate VF endpoints such as ventricular tachycardia (VT) episodes,⁵,¹¹,¹²,¹⁴,¹⁵ ventricular premature beat (VPB) frequency,¹¹,¹³,¹⁴ or electrically induced arrhythmia incidence.⁹,¹² Surrogate endpoints for CAD-related VF may be misleading. In humans, arrhythmias induced by programmed electrical stimulation are now recognized to not predict future VF risk or drug effectiveness,¹⁷,¹⁸ and spontaneous ventricular ectopy can be suppressed by drugs without subsequent suppression of VF.¹⁹ Therefore, CAD-related VF hypothesis-testing using TG mice would be best achieved with a model of VF induced by coronary artery ligation.

In vivo models are inconvenient and are best used for final testing of a well-explored hypothesis. Our aim, therefore, was to develop a mouse perfused heart model to study ischaemia- (and reperfusion-) induced VF as achieved in other species.¹⁰–²²

The strategy was to modify standard Krebs-Henseleit perfusion solution to contain low physiological K⁺ (3 mmol/L) as this facilitates ischaemia-induced VF in animal models,²² just as low blood K⁺ facilitates sudden cardiac death (SCD) in humans.²³ Additionally, the perfusate Ca²⁺ was set high at 2.4 mmol/L as this facilitates animal reperfusion arrhythmias.²⁴ Subsequently, we added potentially pro-arrhythmic endogenous factors ordinarily absent in Krebs solution. We chose catecholamines and angiotensin II, which can facilitate arrhythmias in humans after myocardial infarction,²⁵ and facilitate reperfusion arrhythmias in small animal hearts,²⁶ respectively.

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2. Methods

2.1 General experimental methods

All experiments were performed under Project Licence PPL 70/6635 issued by the United Kingdom Home Office according to the Guide on the Operation of the Animals (Scientific Procedures) Act 1986 which conforms 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). Interventions were randomized, and measurement of variables was blinded.

Male Tuck Ordinary mice (25–30 g) (Bantin and Kingman, Hull, UK) were anesthetized (intraperitoneal pentobarbitone 140 mg kg\(^{-1}\) plus heparin, 3300 units kg\(^{-1}\)), and the heart was excised and arrested in filtered (pore size 5.0 \(\mu\)m) solution (4 \(C\)) containing, in mmol/L: glucose 11.1; CaCl\(_2\) 2.4; NaCl 118.5; NaHCO\(_3\) 25.0; MgSO\(_4\) 1.2; NaH\(_2\)PO\(_4\) 1.21; KCl 3.0; and Cl\(_2\)O (’Krebs’). The aorta was cannulated to a side arm of the aortic cannula. The angiotensin II final test solution at 5% of coronary flow rate via an infusion pump connected to a side arm of the aortic cannula. The angiotensin II final concentration (100 mmol/L) was chosen to be in the middle of a range of EC\(_{50}\) values for angiotensin II’s direct inotropic effects.\(^{30}\) The low volume, close proximity, high concentration angiotensin II-delivery system was designed to minimize the chance of angiotensin II adsorbing on the perfusion apparatus.\(^{22}\)

Drugs were obtained from Sigma-Aldrich (UK), and solutions were made using purified water (ELGA LabWater, UK) with resistivity \(> 18 \text{ M}\Omega \text{cm}\).

2.2 Electrocardiogram recording and analysis

The ECG (PowerLab system, ADInstruments, UK), sampling rate of 2 kHz with high- and low-pass frequency filters set at 0.3 Hz and 1 kHz, respectively, detected arrhythmias defined (at least, in the first instance) according to the Lambeth Conventions.\(^{31}\) During the study the definition of VF was modified. The Lambeth Conventions\(^{31}\) definition of VF was intended to be applicable to single-lead ECG recordings from any model or species: ‘a signal for which individual QRS deflections can no longer be distinguished from one another (implying morphological instability) and for which a rate can no longer be measured’. This definition does not allow for accurate diagnosis of some waveforms seen in the present study, particularly those that appeared to have a dominant frequency and/or were extremely brief (Figure 1). A more precise definition of VF was required, that could be objectively applied and thus avoid the need to rely on subjective judgement. This was achieved by removing ‘a rate can no longer be measured’ from the definition, and specifying a minimum of four consecutive deflections to bring VF in line with the Lambeth Conventions definition of VT. Thus, the final modified definition of VF that we used was ‘a signal for which a minimum of 4 consecutive individual QRS deflections can no longer be distinguished from one another, and for which the rate and morphology of these deflections vary on a beat-to-beat basis, with the variation being neither progressive nor repetitive’.

Hearts in VF were not defibrillated. Any heart in VT or VF at the moment of reperfusion was excluded from the reperfusion data set. VF was additionally quantified using a scoring system: hearts without VF scored zero, those with one episode scored 1, 2–10 episodes scored 2, \(> 10\) episodes scored 3, and hearts with an episode of sustained VF (defined as \(> 2\) min\(^{25}\)) scored 4. The heart rate and PR interval were recorded from the ECG.\(^{24,33}\) QT interval was measured from the beginning of the QRS complex to the point at which the signal reached baseline.

2.4 Statistics

Student’s t-test or analysis of variance followed by Dunnett’s test were used for Gaussian distributed variables (mean \(\pm\) SEM), and Fisher’s exact test was used for binomially distributed variables, with \(P < 0.05\) set as threshold for significance.
3. Results

3.1 Development of exclusion criteria

Preliminary experiments measuring the temperature of the perfusate as it leaves the cannula (no heart attached) showed that temperature is stable at 37°C, and drops transiently after switch of solution by no more than 2°C, with complete recovery to 37°C by 60 s. However, at low flow rates (below 0.5 mL/min) the temperature rapidly dropped below 36°C (Figure 2). Therefore, it was evident that any hearts found to have coronary flow rates below 0.5 mL/min should be excluded, though no hearts in the presented study were excluded on this basis. One heart was excluded because it had an involved zone of 23% and no ischaemia-induced arrhythmias. All other hearts had involved zones within the range 30–61%, so this was considered a preliminary range for inclusion. Hearts displaying unprovoked VF or VT in the 10 min prior to ischaemia should be excluded. No heart was excluded from study for violation of this criterion.

3.2 Catecholamines facilitate ischaemia- and reperfusion-induced ventricular fibrillation

Control hearts did not develop VF at any time during the protocol, but when catecholamines were included in the perfusate both ischaemia and reperfusion were able to elicit VF (both $P < 0.05$ vs. no catecholamines) (Table 1). Angiotensin II did not facilitate ischaemia-induced VF (Table 1), and was therefore not included in subsequent studies. During the execution of this first protocol it became apparent that perfused mouse hearts exhibit high levels of supraventricular arrhythmias (Figure 3).

3.3 Catecholamines facilitate ischaemia-induced ventricular fibrillation through a heart-rate-independent mechanism

To minimize variability and model compromise due to supraventricular arrhythmias, in the next study hearts were paced via the right atrium at 600 b.p.m. At this physiological heart rate (for the mouse34) ischaemia-induced VF remained low in Krebs-perfused hearts, but addition of catecholamines again significantly increased ischaemia-induced VF incidence (Table 2, Figure 4A). Reperfusion-induced VF incidence in paced hearts, however, was high (and statistically not different) in control and catecholamine-perfused hearts (Table 2, Figure 5A), indicating that catecholamines facilitate reperfusion-induced VF at least partly due to the increase in heart rate. Because the highest incidences of ischaemia- and reperfusion-induced VF were achieved when the hearts were paced at 600 b.p.m. and perfused with catecholamines, this set of conditions was chosen as optimal for the model, and its characterization is outlined below.

3.4 Characteristics of ventricular fibrillation

VF never occurred in sham hearts, and occurred only when hearts were subjected to regional ischaemia and/or reperfusion. Susceptibility rose gradually during 30 min ischaemia (Figure 4B). On reperfusion, some hearts displayed VF within 5 min, but a higher incidence was seen during the 5–10 min period (Figure 5B). VF scores for each group were concordant with VF incidence data both in terms of absolute values and temporal distribution of occurrence (Figures 4 and 5).

3.5 Characteristics of other arrhythmias

Ischaemia- and reperfusion-induced VT incidence was similar to VF incidence (Table 2), albeit VT incidence was higher, with some hearts developing VT without the need of perfusion with catecholamines or pacing. VPBs, bigeminy, and salvos were all observed in sham experiments, so those occurring during ischaemia/reperfusion experiments could not be safely deduced to be ischaemia- or reperfusion-induced (Table 2).

3.6 Characteristics of ancillary parameters

Mean involved zone size was 43–50% of ventricular weight, with no differences between groups (Tables 1 and 2). Coronary flow was increased by the addition of catecholamines, but it was not increased (above the control group value) in the involved zone during reperfusion (Table 3). PR interval was shortened by catecholamines (Table 3). QT interval increased during ischaemia and reperfusion compared with the baseline period. Catecholamines significantly increased QT interval prior to ischaemia but had no effect during ischaemia or reperfusion compared with catecholamine-free hearts (Table 3).

4 Discussion

4.1 Optimization of experimental conditions

From this study, we conclude that perfusion with low K+35 and physiological29 supplementation of catecholamines
plus right atrial pacing at the in vivo rate of 600 b.p.m. are sufficient modifications to generate a viable model of ischaemia-induced VF in the isolated mouse heart. The model can express a 57% incidence of VF during ischaemia and a 71% incidence during reperfusion (Table 2, Figures 4A and 5A). To our knowledge, this is the first mouse model exhibiting high levels of ischaemia-induced VF, and as such could be applied to study the role of numerous gene products in mechanisms of VF generation during ischaemia.

Evidence from other isolated heart models suggests that heart rate can influence the rate of development of ischaemia severity and this may contribute to the ability of pacing and catecholamines to facilitate reperfusion-induced VF in the present study.

### Table 1 Ischaemia- and reperfusion-induced arrhythmia incidence data

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Angiotensin II</th>
<th>Catecholamines</th>
<th>Catecholamines + Angiotensin II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Heart rate</td>
<td>380 ± 15</td>
<td>349 ± 23</td>
<td>457 ± 25*</td>
<td>478 ± 22*</td>
</tr>
<tr>
<td><strong>IZ%</strong></td>
<td>50 ± 2</td>
<td>46 ± 2</td>
<td>50 ± 2</td>
<td>48 ± 3</td>
</tr>
<tr>
<td><strong>Ischaemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VF</td>
<td>0/10</td>
<td>0/10</td>
<td>5/10**</td>
<td>5/10**</td>
</tr>
<tr>
<td>VT</td>
<td>3/10</td>
<td>1/10</td>
<td>6/10</td>
<td>5/10</td>
</tr>
<tr>
<td><strong>Reperfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VF</td>
<td>0/10</td>
<td>0/10</td>
<td>5/10**</td>
<td>3/10</td>
</tr>
<tr>
<td>VT</td>
<td>7/10</td>
<td>9/10</td>
<td>9/10</td>
<td>9/10</td>
</tr>
</tbody>
</table>

Incidences of VF and VT. Heart rates and involved zone (IZ) sizes are also shown, displayed as mean ± SEM.

VT, ventricular tachycardia; VF, ventricular fibrillation.

*P < 0.05 vs. control, calculated by one-way ANOVA and Dunnett's post-test.

**P < 0.05 vs. control, calculated by Fisher's exact test.

### Figure 3
Perfused mouse hearts are vulnerable to supraventricular arrhythmias. Electrocardiogram traces from isolated perfused mouse hearts that exhibited relatively frequent episodes of bradycardia and rate irregularity, as well as occasional complete failure of the sinoatrial node.

### 4.2 Implications of findings to mouse in vivo data sets

The present results raise a question about previous in vivo mouse coronary ligation studies. Endogenous catecholamines are present in vivo, yet coronary ligation in the anaesthetized mouse has either failed to evoke VF or has elicited VF in too few animals to permit assessment of suppressive intervention. The reasons for this are not clear. Since we have shown that catecholamines can facilitate the appearance of ischaemia-induced VF in mouse hearts in vitro, an explanation for the apparent inability of endogenous catecholamines to do likewise in vivo requires consideration.

All mouse in vivo coronary ligation studies reported to date were performed in anaesthetized animals undergoing acute surgery. The equivalent acute surgery in rats has been shown to dramatically increase [K⁺]blood. This is important because high cardiac extracellular [K⁺] (in the coronary blood, or the uninvolved region of perfused hearts) can be extremely antiarrhythmic in the setting of acute ischaemia in dog, rat, and rabbit models, and in humans following acute myocardial infarction. Thus, while in the present study we maintained [K⁺] in the uninvolved region at a low physiological level, the previous in vivo studies may have been conducted with [K⁺] blood which was sufficiently high to block ischaemia-induced VF, even in the presence of endogenous catecholamines, although this remains untested.

### 4.3 Characteristics of arrhythmias in the new model

In catecholamine-perfused hearts, VF incidence increased gradually over 30 min of ischaemia (Figure 4). This mirrors the profile of VF in other isolated heart models. On reperfusion, however, the highest incidence of VF was seen 5–10 min after re-admission of flow, whereas in other isolated heart models and in vivo VF is usually initiated within seconds of the start of reperfusion. This may indicate that the balance of pro- and anti-arrhythmic factors is different in the mouse heart compared with other species and that this balance is more pro-arrhythmic at the start of reperfusion in other species than it is in the mouse. As the time profile of VF is not known in humans, it is impossible to say which species is most clinically relevant.

The incidence of VT largely accorded with the incidence of VF, albeit the incidence of VT was generally higher under all conditions. This is reassuring, although the incidence of VT should be viewed as of secondary importance compared with the incidence of VF. The other classes of
Table 2  Arrhythmia data from hearts with supraventricular pacing

<table>
<thead>
<tr>
<th>Incidence of arrhythmia</th>
<th>Before ischaemia</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Catecholamines</td>
<td>Control sham</td>
<td>Catecholamines sham</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Heart rate</td>
<td>600 ± 1</td>
<td>601 ± 1</td>
<td>598 ± 1</td>
</tr>
<tr>
<td>IZ%</td>
<td>44.2 ± 2.2</td>
<td>42.9 ± 2.0</td>
<td>0</td>
</tr>
<tr>
<td>VF</td>
<td>0/13</td>
<td>0/14</td>
<td>0/3</td>
</tr>
<tr>
<td>VT</td>
<td>0/13</td>
<td>1/14</td>
<td>0/3</td>
</tr>
<tr>
<td>Salvo</td>
<td>5/13</td>
<td>4/14</td>
<td>1/3</td>
</tr>
<tr>
<td>Bigeminy</td>
<td>1/13</td>
<td>5/14</td>
<td>0/3</td>
</tr>
<tr>
<td>VPB</td>
<td>12/13</td>
<td>13/14</td>
<td>3/3</td>
</tr>
</tbody>
</table>

Incidences of arrhythmias. Right atria paced at close to 600 b.p.m. Heart rates and involved zone (IZ) sizes are also shown, displayed as mean ± SEM. VT, ventricular tachycardia; VF, ventricular fibrillation; VPB, ventricular premature beat.

*P < 0.05 vs. control, calculated by Fisher’s exact test.

Figure 4  Ischaemia-induced ventricular fibrillation is increased by catecholamines. Analysis of ventricular fibrillation observed during 30 min regional ischaemia in isolated mouse hearts perfused with Krebs containing catecholamines or vehicle control and paced at 600 b.p.m. (A) Incidence of ventricular fibrillation. (B) Incidence of ventricular fibrillation over time. (C) Ventricular fibrillation scores. Each heart was assigned a score based on the severity of the observed ventricular fibrillation (see Methods). (D) Ventricular fibrillation scores over time. *P < 0.05, by Fisher’s exact test.
ventricular arrhythmia were found to occur in sham hearts, so susceptibility during ischaemia and reperfusion cannot be deduced to be a result of ischaemia or reperfusion, meaning that VPBs, bigeminy, and salvos are therefore not valid endpoints in this model.

4.4 Use of an arrhythmia score to analyse initiation and maintenance of ventricular fibrillation

The incidence of VF is the most important endpoint in any model of VF. However, it is useful to take some measurement of the number of episodes of VF, as well as the tendency of VF to sustain. One may count the total number of episodes of VF as well as measuring total VF duration in each heart. However, raw VF durations are not amenable to analysis as continuous variables since VF may not always self-terminate even in small hearts, leading to virtual values of infinity duration, or the need for an arbitrary cut-off duration, both of which violate the requirements for a variable to be Gaussian-distributed. Our approach was to use a VF scoring system. When Curtis and Walker validated a range of scores in a conscious animal model of ischaemia-induced.

Figure 5 Reperfusion can induce relatively high levels of ventricular fibrillation. Ventricular fibrillation observed during 10 min reperfusion, following 30 min regional ischaemia, in isolated mouse hearts perfused with Krebs containing catecholamines or vehicle control and paced at 600 b.p.m. (A) Incidence of ventricular fibrillation. (B) Incidence of ventricular fibrillation over time. (C) Ventricular fibrillation scores. Each heart was assigned a score based on the severity of the observed ventricular fibrillation (see Methods). (D) Ventricular fibrillation scores over time.

Table 3 Coronary flow and electrocardiogram

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Treated</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow (mL/min/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19.8 ± 2.0</td>
<td>21.2 ± 2.4</td>
<td>21.6 ± 2.6</td>
<td>20.2 ± 3.5</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>19.3 ± 1.8</td>
<td>31.2 ± 1.7**</td>
<td>30.9 ± 1.1**</td>
<td>16.3 ± 4.6</td>
</tr>
<tr>
<td>PR interval (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>44.7 ± 1.7</td>
<td>46.7 ± 1.3</td>
<td>47.1 ± 1.2</td>
<td>48.1 ± 2.1</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>44.1 ± 1.3</td>
<td>41.1 ± 2.5</td>
<td>40.9 ± 2.2*</td>
<td>40.6 ± 2.4*</td>
</tr>
<tr>
<td>QT interval (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40.6 ± 3.3</td>
<td>43.5 ± 2.9</td>
<td>61.2 ± 2.5</td>
<td>63.3 ± 4.4</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>44.5 ± 2.7</td>
<td>61.4 ± 3.2**</td>
<td>65.3 ± 2.5</td>
<td>64.0 ± 2.7</td>
</tr>
</tbody>
</table>

Coronary flow per gram of perfused tissue (on reperfusion, reflow per gram of involved tissue is shown) and electrocardiogram intervals. Timing of measurements: Pre-treatment, 1 min before switch to catecholamines/control; Treated, 1 min after switch to catecholamines/control; Ischaemia, after 10 min ischaemia; Reperfusion, after 5 min reperfusion.

*P < 0.05.
**P < 0.01 compared with control.
Calculated by unpaired t-test, with Welch’s correction as necessary.
arrhythmias, they showed that almost any score is valid if it has the property of being hierarchical, (i.e. VF ranked more highly than VT, and so on). The VF score used in the present study is hierarchical, and we have shown that it mirrors VF incidence data both in terms of absolute values and in time-scales (Figures 4 and 5). Although further validation is required, we propose that this scoring system may be useful in analysing VF in future studies.

4.5 Characteristics of ancillary variables

Ischaemic zone size, coronary flow, heart rate, PR interval, and QT interval all appeared to have acceptable levels of variation, with the relationship between mean and standard error being similar to published equivalent relationships for other small animal heart bioassays (e.g. Clements-Jewery et al.,[41]).

In agreement with studies in other isolated heart models,[42] and in humans,[43] catecholamines shortened PR interval and increased coronary flow rates (Table 3). A catecholamine-induced increase in coronary flow was not seen in reperfused tissue, however, which we postulate is due to catecholamine-induced direct coronary vasoconstriction offsetting catecholamine-induced endothelium-dependent vasodilatation. Direct vasoconstriction (e.g. via α1 adrenoceptor activation of vascular smooth muscle) may be favoured during reperfusion due to the endothelial damage that has been shown to occur in reperfused isolated hearts.[41]

The QT interval increased during ischaemia as it does in isolated rat, rabbit, and primate hearts.[44,45] QT prolongation ordinarily indicates delayed repolarization, and in humans is often caused by reduced IKr.[46] In mice, however, IKr is not a major repolarizing current. Instead, IKs,slow 1, IKs,slow 2, and IKs are the predominant K+ currents.[44] Thus, QT measurements in mouse hearts cannot be directly extrapolated to human. There is a practical problem with measuring QT in the mouse heart as there is no obvious T wave in the ECG.[47,48] This means that it is difficult to measure the point at which repolarization ends accurately, and published results vary.[49–51] Therefore, the value of QT measures in the isolated mouse heart remains to be validated.

4.6 Future applications of the model

The most useful application of this model and, indeed, functional genomics in general, will be in studying potential therapeutic targets for which there are no selective drugs. There are already many strains of mice available that have manipulations of genes/gene products that are altered during ischaemia, and the new model can be used to test their involvement in arrhythmogenesis. A particularly important future use would be to study human genetic variations identified by association studies as being linked to coronary artery disease-related deaths.

In conclusion, perfusion with physiological levels of catecholamines and right atrial pacing at 600 b.p.m. converts the VF-resistant mouse heart into a viable bioassay for studying VF mechanisms in ischaemia and reperfusion using functional genomics.

Acknowledgements

Abigail Rickard, Ellen Andrag, Andras Farkas, Mike Shattock, and Metin Avkiran are thanked for support and advice.

Conflict of interest: none declared.

Funding

The work was supported by the British Heart Foundation [FS/05/023].

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