Arrhythmogenesis in experimental models of heart failure: the role of increased load

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Received 19 September 1995; accepted 15 February 1996

Abstract

Objectives: To assess the effects of cardiac failure due to doxorubicin cardiotoxicity or chronic myocardial infarction on arrhythmia induction, ventricular repolarization and refractoriness in isolated perfused rabbit hearts under different loading conditions. Methods: Cardiac failure was induced by doxorubicin injection (1–1.25 mg·kg⁻¹ twice weekly for 8 weeks, n = 16) or coronary ligation (n = 12), with 12 controls. Cardiac failure was defined by an echocardiographic ejection fraction ≤ 0.40. Arrhythmia susceptibility was assessed by programmed ventricular stimulation and fibrillation threshold measurement during Langendorff and during working heart perfusion under baseline conditions and at maximum tolerated preload and afterload. Monophasic action potential duration, dispersion of refractoriness, conduction time and effective refractory period were measured at each level of load. Results: During unloaded (Langendorff) perfusion, there was a low incidence of arrhythmia induction in all hearts. Increasing load did not alter arrhythmogenesis significantly in normal hearts, but led to increases in arrhythmia inducibility and falls in fibrillation threshold which were significantly greater in failing than in non-failing hearts. Monophasic action potential duration was significantly (P < 0.05) shorter in failing than in non-failing hearts in the doxorubicin-treated [mean (s.e.m.) 140(2) vs. 147(2) ms] and post-infarction groups [146(2) vs. 154 (3) ms] during working heart perfusion. The shortening in action potential duration and effective refractory period during increased preload tended to be greater in failing than in non-failing hearts. There were no changes in conduction times in response to changes in loading. Conclusions: The inducibility of ventricular arrhythmias is greater in failing than in non-failing hearts and is further enhanced by increases in preload. Shortening of repolarization and refractoriness due to increased preload may contribute to the increased risk of ventricular tachyarrhythmias and sudden death in cardiac failure.

Keywords: Heart failure; Sudden death; Monophasic action potential; Ventricular fibrillation; Arrhythmias; Doxorubicin; Rabbit, heart

1. Introduction

Congestive heart failure is a condition associated with a poor prognosis and a particularly high risk of sudden cardiac death [1]. The commonest cause of sudden death is thought to be ventricular tachycardia or fibrillation although it is recognized that bradyarrhythmias or asystole may be an important factor in advanced heart failure [2]. Patients with poor ventricular function and malignant ventricular arrhythmias have a high incidence of inducible ventricular tachycardia or fibrillation, and respond poorly to conventional antiarrhythmic drug therapy [3]. Multivariate analysis has identified an elevated left ventricular preload, measured as increased pulmonary capillary wedge pressure, as an independent predictor of sudden death in heart failure [4].

There are numerous potential arrhythmogenic mechanisms in heart failure including acute ischaemia, scar-related re-entry, cellular electrophysiological changes, increased catecholamine levels, electrolyte abnormalities and contraction–excitation feedback [5]. Some of these may be disease-specific (e.g., acute ischaemia), but it is noteworthy that the prognosis and the risk of sudden death are similar in advanced heart failure of different aetiologies [6].

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PII S0008-6363(96)00080-6
The objective of the current study was to evaluate changes in arrhythmia inducibility, myocardial repolarization, refractoriness and conduction time in experimental heart failure and to determine the relationship between the extent of left ventricular dysfunction and electrophysiological changes during stable in vitro perfusion and in response to acute changes in preload and afterload.

2. Methods

All procedures were undertaken in accordance with the Animals (Scientific Procedures) Act 1986.

2.1. Doxorubicin cardiomyopathy

A well-documented regimen was adopted for doxorubicin-induced heart failure in rabbits [7-9]. Adult male New Zealand White rabbits weighing 2.5-3.5 kg were used for the study. Doxorubicin was administered via a marginal ear vein in a dose of 1-1.25 mg kg⁻¹ twice weekly for 8 weeks. Control rabbits received 0.9% saline in equivolumetric doses over the same period.

2.2. Coronary ligation

Adult New Zealand White rabbits (2.5-3.5 kg) received premedication with intramuscular fentanyl/fluanisone (Hypnorm, Jansen Pharmaceuticals) 0.4 ml kg⁻¹. Anaesthesia was induced with midazolam (1-2 mg kg⁻¹) given via an indwelling cannula in the marginal ear vein. The rabbit was intubated and ventilated using a Harvard small animal ventilator with a mixture of nitrous oxide, oxygen and halothane in a 1:1:1 ratio at a tidal volume of 20 ml and a frequency of 50 min⁻¹. A left thoracotomy was performed through the 4th intercostal space. Quinidine hydrochloride 10 mg kg⁻¹ (Sigma Pharmaceuticals) was administered intravenously 15 min prior to coronary artery ligation to reduce the incidence of ventricular fibrillation. The major ventricular branch of the left coronary artery was ligated halfway between its origin and the cardiac apex in order to produce an area of ischaemia of 20-25% of the left ventricle. Ventricular fibrillation occurred in approximately 30% of cases, usually 8-12 min following occlusion. Defibrillation was undertaken with a 2.0 joule epicardial shock. If repeated fibrillation occurred, the ligation was released and restituted distally. When an acceptable area of infarction had been produced and the animal was haemodynamically and electrically stable, the thoracotomy was closed. The animals were given intramuscular antibiotics for 48 h, and received 20 ml of isotonic saline subcutaneously to replace perioperative losses. Postoperative analgesia with buprenorphine 0.03 mg kg⁻¹ every 8 h [10] was continued for the first 3-4 days, combined with convalescence in a warm clean environment with adequate monitoring of any distress.

2.3. Echocardiographic assessment of left ventricular function

Echocardiographic examination was performed after 10 weeks in the doxorubicin-treated group and after 8 weeks in the ligation group. Under light fentanyl/fluanisone sedation, the animal was placed prone on a table with an area removed so that the ultrasound probe, a Toshiba 5 MHz neonatal transducer, could be brought from below and placed on a shaved area of the anterior chest wall. A Toshiba SSH160A echocardiograph with 2-dimensional real time and M-mode acquisition capabilities and on-line cine loop computer analysis facilities was used for all examinations. Left ventricular ejection fraction was assessed from a short axis image just below the tips of the mitral valve. Using the cine loop facility, the end-diastolic and end-systolic frames were captured and the endocardial border, excluding the papillary muscles, was traced onto the screen. The enclosed area was automatically computed, and the ejection fraction (area) was calculated as: end-diastolic area - end-systolic area/end-diastolic area.

2.4. In vitro study

2.4.1. Perfusion technique

Rabbits were injected with heparin 2000 IU intravenously and then given an overdose of sodium pentobarbitone. The heart was removed and placed in a beaker containing oxygenated Tyrode’s buffer chilled to 4°C. After cannulation of the aorta, retrograde perfusion by the Langendorff technique [11] was started, one of the pulmonary veins was then cannulated, and the others were ligated. After equilibration and baseline measurement of coronary flow, perfusion in the working heart mode [12] was initiated by opening the left atrial inflow and allowing left ventricular ejection. The height of the left atrial reservoir (preload) was set at 0-10 cm H₂O, with an aortic column of 80-100 cm H₂O (afterload) against which the left ventricle ejected. The perfusate and a water-jacketed glass chamber around the heart were heated and thermoregulated to maintain the temperature of the epicardium at 34-35°C. The perfusion medium was a modified Tyrode’s buffer solution pH 7.40, equilibrated with O₂/CO₂ (95:5). The final concentrations in this buffer were (mM): Na⁺ 142, K⁺ 4.0, Ca²⁺ 1.8, Mg²⁺ 1.0, H₂PO₄⁻ 0.4, HCO₃⁻ 28, glucose 11.0. The perfusion fluid was renewed every hour during the procedure to ensure an adequate glucose concentration. Aortic flow in working hearts was measured using a calibrated low-resistance flow meter, and coronary sinus flow was measured by timed collection of the effluent from the right ventricle. Cardiac output was calculated as the sum of aortic and coronary sinus flow. Samples of coronary effluent were collected during the experiment for later estimation of lactate concentration using a commercially available enzymatic method (Boehringer Mannheim Diagnostica).
Left ventricular pressure was recorded from a 21-gauge short plastic cannula inserted by direct puncture, and attached via stiff wide-bore plastic tubing to a Gould pressure transducer and the Mingograph 7 inkjet recorder. At the stimulation frequency used, and particularly in the failing hearts, the relationship between left atrial reservoir height and left ventricular end-diastolic pressure was not linear. Left ventricular end-diastolic pressure was used as the index of left ventricular preload.

2.5. Monophasic action potentials

Monophasic action potentials (MAP) were recorded simultaneously from the anterior left ventricle, apex and base using Ag/AgCl suction electrodes and amplified by a 3-channel high-input impedance differential amplifier with a frequency response from DC to 1.25 kHz. The amplified MAP signals were displayed on a Gould 1420 digital storage oscilloscope and recorded for subsequent analysis on to a videocassette using a Medical Systems Corporation PCM-8 analogue to digital (A/D) videocassette recorder adapter with a 3 dB frequency response of DC to 3500 Hz.

Analysis of monophasic action potentials was undertaken off-line using an analysis package based on an IBM-compatible microcomputer equipped with an analogue-to-digital converter board and interface unit. The sampling frequency of the action potential data was 1 kHz. The system provided real-time graphic display of the monophasic action potentials with digital measurement of the time from the stimulus to the beginning of the rapid upstroke of the monophasic action potential and the action potential durations at 50 and 90% repolarization.

2.6. Effective refractory period

Three pairs of platinum plunge/hook electrodes were placed superficially in the left ventricular myocardium within 1 mm of each of the adjacent monophasic action potential electrodes. The effective refractory period (ERP) at each site was determined at twice diastolic threshold by bipolar stimulation using a constant current impulse generator (DS7 Digitimer Ltd) driven by a computer-based stimulation programme. An 8-cycle drive train was delivered to the ventricle at a cycle length of 300 ms followed by a timed extra stimulus delivered after 190 ms, with a 4 s pause before the next drive train. The extra stimulus interval was reduced in 5 ms steps until loss of capture. This interval was defined as the effective refractory period.

2.7. Conduction and repolarization times

MAP's were recorded during pacing (cycle length 300 ms) at each of the 3 left ventricular stimulation sites. Local conduction times were measured as the time from the stimulus artefact to the initial rapid deflection of the recorded MAP upstroke. Repolarization time was measured during right atrial pacing as the interval from the stimulus artefact to the 90% repolarization point of the MAP signals. Dispersion of repolarization was measured as the maximum difference between the repolarization times of the 3 simultaneously recorded MAP's.

2.8. Programmed ventricular arrhythmia induction

Arrhythmia induction was attempted by programmed ventricular stimulation using an 8-beat drive cycle at 300 ms followed by up to 3 extrastimuli, with a 4 s pause between each drive train. Stimulation was undertaken at two ventricular sites, normally those with the shortest effective refractory periods but including one site adjacent to the infarct border zone in the chronic infarction hearts. The endpoint of ventricular stimulation was sustained ventricular tachycardia or fibrillation lasting > 30 s, or completion of the full protocol at both stimulation sites.

2.9. Ventricular fibrillation threshold

Ventricular fibrillation threshold was determined using a train of 10 constant current impulses of 4 ms duration at 10 ms intervals via the bipolar platinum stimulating electrodes in the epimyocardium, during right atrial pacing at a 300 ms cycle length. The train of impulses was started 50 ms before the effective refractory period (ERP) and therefore continued for about 50 ms after the ERP, to ensure that one of the impulses captured the ventricle during the vulnerable period. The current delivered to the ventricle was increased in 5 mA increments until fibrillation ensued (ventricular fibrillation threshold) or a maximum of 100 mA was delivered. Following fibrillation the heart was electrically defibrillated. Individual determinations of the VF threshold were made at intervals of not less than 7 min.

2.10. In vitro haemodynamic and electrophysiology protocol

After isolation and stabilization of the preparation, the heart was paced from the right atrium at a cycle length of 300 ms. The lowest preload necessary to achieve a stable aortic flow of between 80–90 ml/min against an afterload of 75 cmH2O during working heart perfusion was determined and defined as the baseline. Increased preload was determined by raising the level of the left atrial filling reservoir height until the maximum stable aortic flow was achieved. Increased afterload was defined as the maximum aortic ejection line height which could be maintained without the development of an acute fall in forward aortic flow. Exposure to increased preload or afterload was maintained for a minimum of 5 min. The same increased preload and afterload was used throughout each particular experiment. Measurements of left ventricular peak systolic
and end-diastolic pressure, aortic and coronary sinus flow were made under each loading condition.

Monophasic action potentials and effective refractory periods were recorded in the Langendorff, baseline working heart, increased preload and increased afterload perfusion states, returning to baseline working heart conditions after each change in load. The same MAP sites were used throughout the experiment, but the MAP electrode was re-applied after each manoeuvre to achieve high-quality MAP signals. Thirty seconds was allowed after changing load for stabilization before MAP signals were recorded. The same MAP records were used to assess activation time, dispersion of repolarization and action potential duration. During the initial experiments, a separate MAP recording was made with the electrode re-applied at high loading conditions at the same site to exclude a change in electrical contact as a cause of any signal change. Acceptable reproducibility was taken as a difference in MAP duration of less than 3 ms.

Following the initial recordings of MAP's and effective refractory periods under different loading conditions, the MAP suction electrodes were removed, and the loading protocol was repeated with programmed ventricular stimulation and ventricular fibrillation threshold determination during Langendorff and baseline working heart perfusion and following increased preload and increased afterload.

2.11. Statistical methods

Continuous data are presented as mean ± standard error of the mean. Student's two-sample paired or unpaired t-tests were applied for normally distributed data, and the non-parametric method of Mann-Whitney was used for skewed data. A chi-squared test for discontinuous variables was used with a Fisher exact correction introduced for small numbers where the observed or expected frequency was less than 5. Analysis of variance was applied for multiple comparisons of related data between groups (parametric ANOVA for normal data and Kruskal-Wallis analysis of variance for non-parametric data). When a significant difference was indicated by the F-statistic, the Newman-Keuls multiple comparison test was applied to identify differences in mean values. For continuous comparison between grouped data, correlation coefficients (r) were estimated for the degree of association between two variables. If these were found to be significant, then scatter plots of the two sets of data were made and simple regression analysis was performed. A value of P < 0.05 was taken as statistically significant.

3. Results

3.1. Mortality and signs of cardiac failure

The total mortality in the coronary ligation group was 35%, primarily occurring at the time of coronary ligation or in the first 48 h postoperatively. The myocardial infarctions in the coronary ligation group were all transmural on histological examination. The mean extent of infarction of the left ventricle as measured by planimetry of 4 short-axis slices stained with haematoxylin and eosin was 25% (range 11–47%).

Evidence of overt congestive cardiac failure (pleural effusion, ascites or scrotal oedema) was noted in 6/7 doxorubicin-treated and 4/6 coronary ligation animals with ejection fractions ≤ 0.4, but in none of the controls or in doxorubicin-treated or coronary ligation animals with

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>In vivo and in vitro haemodynamic data</strong></td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Doxorubicin</td>
</tr>
<tr>
<td>≤ 0.4</td>
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<tr>
<td>Infarction</td>
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<td>≤ 0.4</td>
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</tbody>
</table>

Values are means (s.e.m.). * P < 0.05, † P < 0.01, doxorubicin/infarction EF > 0.4 vs. normal. ++ P < 0.05, ++ P < 0.01, EF > 0.4 vs. EF ≤ 0.4.

EF = ejection fraction area; LVSP = left ventricular systolic pressure (mmHg); CO = in vitro cardiac output (ml·min⁻¹); LVEDP = left ventricular end-diastolic pressure (mmHg).
ejection fractions > 0.4. The doxorubicin-treated and coronary ligation groups were subdivided according to ejection fraction (> or ≤ 0.4) into preserved left ventricular function (non-failing) and impaired function (failing) groups.

3.2. Haemodynamic data

The in vivo and in vitro haemodynamic data are listed in Table 1. There was no significant difference between the sham-operated and saline-injected controls in any haemodynamic or electrophysiological parameter, and therefore the data have been pooled. There were only minor differences at baseline between the cardiac output and end-diastolic pressures in controls and in the doxorubicin-treated or post-infarction hearts with ejection fractions > 0.40 (Table 1). In contrast, the mean cardiac outputs were lower and the end-diastolic pressures higher in the hearts with ejection fractions ≤ 0.40. An increase in preload produced an increase in cardiac output in all hearts, but the response was suboptimal in the infarcted hearts with preserved ejection fraction and in both groups of failing hearts. The increase in left atrial reservoir height resulted in an increase in left ventricular end-diastolic pressure in all groups, but this was particularly marked in the failing hearts.

The peak afterload tolerated by the hearts was lower in those with impaired ejection fraction. All groups demonstrated a similar reduction in cardiac output in response to increased afterload, but failing hearts showed a greater increase in end-diastolic pressure than non-failing hearts.

There was no significant change in baseline haemodynamic data following each exposure to increased preload or afterload for the duration of the experimental protocol. No significant evidence of coronary sinus lactate production was found during baseline perfusion or exposure to increased preload or afterload.

3.3. Spontaneous ventricular arrhythmias

Occasional ventricular premature beats were recorded after acute changes in load, more usually in preload, in all

Fig. 1. Inducibility of sustained ventricular arrhythmias under different loading conditions. BLWH = baseline working heart. * P < 0.05, ** P < 0.01, EF ≤ 0.40 vs. EF > 0.40.

Fig. 2. Mean ventricular fibrillation thresholds under different loading conditions. BLWH = baseline working heart. * P < 0.05, ** P < 0.01, EF ≤ 0.40 vs. EF > 0.40.
groups. These extrasystoles were infrequent and often not reproducible, with no obvious differences between groups. No episodes of spontaneous ventricular tachycardia or fibrillation occurred in response to changes in load.

3.4. Ventricular arrhythmia induction

The arrhythmias induced by programmed stimulation were polymorphic ventricular tachycardia or ventricular fibrillation. Under unloaded (Langendorff) conditions, no sustained ventricular arrhythmia could be induced in the normal hearts (Fig. 1), and in only one of the doxorubicin and ligation hearts with preserved left ventricular function. Among the failing hearts, arrhythmias were more commonly induced, in 3/7 (43%) of the doxorubicin and 2/6 (33%) of the ligation hearts. The change to working heart mode and subsequently to increased preload or afterload resulted in induction of arrhythmias in a maximum of 2/12 hearts in the normal group. Of the diseased hearts, 33% of the group with preserved left ventricular function became inducible with increasing preload, in contrast to over 80% of the failing hearts (P < 0.01). The proportion of failing hearts with inducible arrhythmias was already so high in baseline working heart mode that the only further change noted with increased pre- or afterload was in response to increased afterload in the infarction group.

3.5. Ventricular fibrillation threshold

The mean ventricular fibrillation threshold was high (95–100 mA) under all loading conditions in normal hearts (Fig. 2). There were no significant differences in ventricular fibrillation threshold between experimental groups during Langendorff perfusion. The change to baseline working heart perfusion resulted in a significant reduction in ventricular fibrillation threshold in both the failing and non-failing groups of doxorubicin-treated and coronary ligation hearts, but not in normal hearts. Further increase in preload produced additional reductions in fibrillation threshold in the failing hearts (P < 0.01). There was no significant change in fibrillation threshold in response to increased afterload compared with baseline working heart mode in any experimental group.

3.6. Stability of electrophysiological measurements

Monophasic action potential duration in baseline working heart mode was measured after stabilization following each exposure to increased load. The mean MAPD₀ after 60 min of the experimental protocol was 98 ± 1% of the initial value. Following each application of the suction electrode, the monophasic action potential amplitude and plateau began to deteriorate slowly, requiring re-application after approximately 4 min. The signal amplitude often fell during increases in load. MAP signals were accepted for analysis if the amplitude was > 15 mV and the beat-to-beat standard deviation of MAPD₀ did not exceed 2 ms. If these criteria were not met, the suction electrode was removed, replaced at an identical site, and the manoeuvre was repeated.

3.7. Monophasic action potential duration

The mean values of MAPD₀ in the different experimental groups under various loading conditions are listed in Table 2. There was no significant difference in MAPD₀ under unloaded (Langendorff) conditions between the normal hearts and the doxorubicin-treated or post-infarction hearts, irrespective of ventricular function. Change from Langendorff to working heart perfusion caused a significant shortening in MAPD₀ in all groups except the doxorubicin-treated failing hearts, while further increase in preload resulted in further significant shortening of MAPD₀ in all groups except the normals. Under baseline working heart conditions, the mean values for MAPD₀ in the doxorubicin-treated and post-infarction hearts with preserved ventricular function were significantly longer than in normal hearts. In contrast, the MAPD₀ in the hearts with impaired left ventricular function was significantly shorter than in the hearts with preserved ventricular function in both the doxorubicin and post-infarction groups. This difference between hearts with preserved and impaired ventricular function was also seen under increased preload. The overall extent of shortening from the unloaded Langendorff state to maximum preload was not significantly different between experimental groups (approximately 15 ms or 10%) with the exception of the failing post-infarction hearts in which a mean shortening of 27 ms was recorded.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Langendorff</th>
<th>BLWH</th>
<th>Max. preload</th>
<th>Max. afterload</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12</td>
<td>149 *</td>
<td>136</td>
<td>134</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5)</td>
<td>(3)</td>
<td>(4)</td>
<td>(3)</td>
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<tr>
<td>Doxorubicin</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>EF &gt; 0.4</td>
<td></td>
<td>154 *</td>
<td>147 *</td>
<td>140 *</td>
<td>148 +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
</tr>
<tr>
<td>EF ≤ 0.4</td>
<td></td>
<td>146</td>
<td>140</td>
<td>131</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td>(2)</td>
<td>(3)</td>
<td>(3)</td>
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<tr>
<td>Infarction</td>
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<td></td>
</tr>
<tr>
<td>EF &gt; 0.4</td>
<td></td>
<td>163 *</td>
<td>154 *</td>
<td>145 *</td>
<td>156 +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4)</td>
<td>(3)</td>
<td>(2)</td>
<td>(3)</td>
</tr>
<tr>
<td>EF ≤ 0.4</td>
<td></td>
<td>160 *</td>
<td>146</td>
<td>133</td>
<td>147 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

Values are mean (s.e.m.) of monophasic action potential duration (ms) at 90% repolarization from 3 ventricular sites in each heart.

* P < 0.05, * * P < 0.01, vs. baseline working heart mode.

* * P < 0.05, * * * P < 0.01, doxorubicin/infarction EF > 0.4 vs. normal.

† P < 0.05, † † P < 0.01, EF > 0.4 vs. EF ≤ 0.4.

BLWH = baseline working heart.
There was no significant change in MAPD₉₀ between baseline working heart mode and maximum afterload in any experimental group. The values in the doxorubicin-treated and post-infarction hearts with preserved ventricular function remained significantly longer than in normal hearts, while MAPD₉₀ in the post-infarction hearts with impaired ventricular function was significantly shorter than in those with preserved function.

There was no significant change in dispersion of repolarization in response to changes of loading in any experimental group, or any difference between the failing and non-failing hearts in the doxorubicin or post-infarction groups (Table 3). The post-infarction hearts with ejection fraction < 0.4 showed significantly greater dispersion of repolarization than in normals under all loading conditions. There was significantly greater dispersion of repolarization in the post-infarction hearts than in the doxorubicin-treated hearts during baseline working heart perfusion.

3.8. Effective refractory period

The data for effective refractory period in the experimental groups closely paralleled the values for monophasic action potential duration both between experimental groups and in response to changes in loading. There was no significant change in the relationship between monophasic action potential duration and effective refractory period in any group or under any loading condition (data not shown).

3.9. Conduction times

There were no significant changes in mean conduction times in response to changes in loading in any experimental group, or any differences between groups (data not shown).

3.10. Correlations between electrophysiological and haemodynamic findings

The results presented thus far have indicated many similarities between the electrophysiological findings in the doxorubicin cardiomyopathy and post-infarction groups. Although differences in dispersion of repolarization were demonstrated (Table 3), these did not correlate with changes in arrhythmia inducibility or ventricular fibrillation threshold. The data suggest that the easier arrhythmia induction in the failing hearts may be determined as much by the degree of left ventricular dysfunction as by the specific mechanism of cardiac damage. To investigate the inter-relationships between haemodynamic function and electrophysiology in more detail, the normal, doxorubicin-treated and coronary ligation data were pooled, and correlations between haemodynamic and electrophysiological data were sought. A weak correlation (r = 0.38, P = 0.017) was found between in vivo ejection fraction and the extent of MAPD₉₀ shortening on increasing preload. There was a closer correlation between ejection fraction (r = 0.79, P < 0.001, Fig. 3) and ventricular fibrillation threshold during working heart perfusion. Thus left ventricular contractile dysfunction was associated with a lower ventricular fibrillation threshold.

There were significant negative correlations between the changes in ventricular fibrillation threshold, MAPD₉₀, and ERP and the change in cardiac output from Langendorff to working heart mode: i.e., the greater reduction occurred in the hearts with the smaller increase in cardiac output (Table 4). A similar relationship was seen for changes in MAPD₉₀ and ventricular fibrillation threshold on increasing preload. Significant positive correlations were also found between the increase in left ventricular end diastolic pressure and reductions in ventricular fibrillation threshold, MAPD₉₀ and ERP on increasing preload. There were no significant correlations between electrophysiologi-
Table 4
Correlations between haemodynamic and electrophysiological changes during loading

<table>
<thead>
<tr>
<th>ΔCardiac output</th>
<th>ΔLVEDP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lang/BLWH BLWH/MaxPre Lang/BLWH BLWH/MaxPre</td>
<td></td>
</tr>
<tr>
<td>ΔVFT mA</td>
<td>0.41 *</td>
</tr>
<tr>
<td>ΔMAPD_s0 ms</td>
<td>0.27 *</td>
</tr>
<tr>
<td>ΔERP</td>
<td>0.35 * *</td>
</tr>
</tbody>
</table>

Δ Cardiac output = change in cardiac output; ΔLVEDP = change in left ventricular end-diastolic pressure; Δ VFT = reduction in ventricular fibrillation threshold; Δ MAPD_s0 = maximum reduction in each heart of MAPD_s0 during increased loading; Δ ERP = maximum reduction in each heart of ERP during increased loading; Lang = Langendorff perfusion; BLWH = baseline working heart perfusion; MaxPre = maximum preload.

* P < 0.05, ** P < 0.01, *** P < 0.001.

cal parameters and changes in cardiac output and LVEDP in response to increased afterload.

4. Discussion

The inducibility of sustained ventricular arrhythmias in the present study was assessed by two separate techniques—programmed ventricular stimulation and measurement of ventricular fibrillation threshold. The results obtained by the two techniques were consistent. The inducibility of arrhythmias in normal hearts was low, even under increased loading conditions. Some previous studies have demonstrated no effect of load on spontaneous or induced arrhythmias in normal hearts [13–15], while others have found that increased preload either made arrhythmias more easily inducible or lowered ventricular fibrillation threshold [16,17]. The explanation for these discrepancies may lie in different rates and extent of volume stress.

Differences in arrhythmia inducibility or ventricular fibrillation threshold between the normal and doxorubicin-treated or coronary ligation hearts in the present study were minimal under unloaded conditions, but became progressively more marked in response to increasing load. Inducibility of arrhythmias was consistently and significantly greater in the failing doxorubicin-treated and post-infarction hearts than in the corresponding non-failing groups in working heart mode and under increased preload. The association between left ventricular function and arrhythmia inducibility is supported by the overall correlation between ejection fraction and ventricular fibrillation threshold (Fig. 3).

The results presented in this study demonstrate that failing hearts have shorter left ventricular action potential durations and effective refractory periods than non-failing hearts during working heart perfusion, and that this effect tends to increase with increasing preload, suggesting greater contraction-excitation feedback. The differences in repolarization and refractoriness may account in part for the greater ease of arrhythmia induction in the failing hearts. Analysis of the electrophysiological mechanisms responsible for the arrhythmias induced in the experimental models of heart failure is beyond the scope of the present study. Shortening of refractoriness in the absence of significant change in conduction velocity will decrease the wavelength in a potential re-entry circuit and thus favour the chances of re-entry [18]. Shortening of repolarization and refractoriness in failing hearts under conditions of increased load would make triggered activity due to early afterdepolarizations unlikely, although delayed afterdepolarizations are a possible arrhythmogenic mechanism. Preliminary data in the rabbit coronary ligation model indicates a paradoxical increase in sarcoplasmic reticulum Ca²⁺ uptake [19], in contrast to a decrease seen in preparations from explanted human hearts at transplantation [20]. Spontaneous Ca²⁺ release from the sarcoplasmic reticulum has been observed, and may contribute to arrhythmogenic inward currents [19]. The magnitude of the differences in repolarization and refractoriness between the failing and non-failing hearts in this study was small, as was the dispersion of repolarization. More extensive mapping, and recordings from intramyocardial and endocardial sites, might have demonstrated greater differences in repolarization. Furthermore, there may be greater heterogeneity in repolarization and refractoriness following premature beats, as has been demonstrated in man [21]. It should be noted that the hypothetical association between action potential shortening and arrhythmia inducibility is not supported by the data during increased afterload, where the differences of repolarization and refractoriness between the failing and non-failing hearts are less impressive or non-significant, despite the persisting differences in arrhythmia inducibility.

The literature on changes in repolarization and refractoriness due to heart failure is confusing, with the majority of authors [22–24] reporting an increase in action potential duration and refractory period, although some [9,25] report a shortening. There are many possible explanations for these discrepancies, including differences of species and cause of heart failure. It is noteworthy that most studies showing prolonged action potential duration were performed at unphysiologically long cycle lengths. Our data, obtained at a physiological heart rate, indicate that the severity of left ventricular dysfunction is an important determinant of myocardial repolarization. There was a tendency for action potential duration in the non-failing doxorubicin-treated and infarction hearts to be longer than in controls, consistent with the effects of compensatory hypertrophy [5,26,27]. In contrast, repolarization and refractoriness were shorter in the two failing heart groups.
than in the corresponding non-failing groups during baseline working heart perfusion or increased preload. The absence of significant differences in repolarization and refractoriness between normal and diseased hearts or between failing and non-failing hearts under Langendorff perfusion suggests that studies performed under unloaded conditions, including possibly those on isolated myocytes, may be insufficiently sensitive to detect differences in repolarization and refractoriness between failing and non-failing hearts.

In common with other authors [14,28] we have found shortening in action potential duration to be greater in response to increase in preload than to increase in afterload. Although there was a weak but significant inverse correlation between ejection fraction and shortening of action potential duration on increasing preload, the magnitude of the difference in mechano-electrical feedback between healthy and diseased or failing hearts appeared to be small. This observation is in variance with previous reports of greater contraction–excitation feedback in hearts following coronary ligation than in controls [29–31]. Previous studies have used larger, possibly unphysiological, changes in load to demonstrate contraction–excitation feedback [9,11]. The increases in preload and afterload in our study were 'physiological' in that the hearts were haemodynamically and biochemically stable during increased load, and the changes were reversible on returning to baseline.

The absence of a significant effect of load on conduction times in the present study is in agreement with previous work indicating a lack of effect during regular pacing [16,32] or ventricular tachycardia [18]. We cannot exclude the development of regional heterogeneity of conduction velocity in response to premature stimuli, which might be enhanced in failing hearts or under increased load and might predispose to arrhythmogenesis.

The findings of this study, if extrapolated to the clinical setting, would imply that the risk of lethal ventricular arrhythmias in patients with heart failure may be greater during periods of fluid retention and consequent increase in ventricular preload. Such an association is difficult to establish, since the definition of 'sudden death' commonly employed in natural history studies or clinical trials in heart failure [33,34] normally excludes cardiac arrests occurring at times of haemodynamic deterioration. Extrapolation of our data must be made with caution, since inducibility of sustained ventricular tachyarrhythmias in patients with cardiac failure is not predictive of sudden death [4], although persistent arrhythmia inducibility in patients with a history of cardiac arrest and left ventricular dysfunction is an adverse prognostic sign [3]. It is of interest that pulmonary arterial wedge pressure, an indirect measure of left ventricular preload, is an independent predictor of sudden death in patients with heart failure [4]. Thus prevention of increases in left ventricular preload or dilatation may be an important target in the prevention of sudden death in heart failure.

Acknowledgements

Dr. Maurice Pye was supported by a British Heart Foundation Junior Research Fellowship.

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