Effect of sustained stretch on dispersion of ventricular fibrillation intervals in normal rabbit hearts

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Abstract

**Objective:** To determine the effect of acute left ventricular dilatation on refractoriness in normal hearts. **Methods:** During sustained ventricular fibrillation (VF) in isolated perfused hearts, recording of local activation time yields VF intervals which provide an index of local refractoriness. Simultaneous measurement from multiple sites enables study of spatial aspects of changes in refractoriness. We studied the effects of stretch on the magnitude and dispersion of changes in VF interval in 10 isolated, Langendorff-perfused rabbit hearts using a flexible epicardial array containing 240 unipolar electrodes. The left ventricular pressure was increased from 0 to 40 mmHg by inflation of an intraventricular balloon during sustained VF. **Results:** The current threshold for VF induction fell from 64±11 mA to 43±11 mA (mean±SE, P<0.01) following ventricular dilatation. Mean VF interval at 0 mmHg was 79.8±1.3 ms and fell to 70.2±1.7 ms (P<0.01) at 40 mmHg. There was a corresponding increase in dispersion of VF interval (coefficient of variation) from 8.13±0.8 to 13.3±0.8 (P<0.01). There was regional heterogeneity in the areas of greatest reduction in VF interval, which varied between hearts. Following balloon inflation there was an increase in the number of activation waves. **Conclusions:** Acute ventricular dilatation produces spatially heterogeneous changes in refractoriness which would predispose to the maintenance of reentrant arrhythmias.

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1. Introduction

Patients with congestive heart failure or significantly impaired left ventricular function are at high risk of sudden cardiac death, frequently due to malignant ventricular arrhythmias [1,2]. Numerous factors predispose to arrhythmias, including increased sympathetic tone, ionic disturbances, ischaemia and structural abnormalities associated with infarction and fibrosis [3]. However, alterations in ventricular size, pressure or function are common to all types of congestive heart failure regardless of aetiology, and the degree of mechanical dysfunction is a good predictor of the risk of sudden death [4]. Evidence of a potential role for mechanoelectrical feedback in the initiation and maintenance of arrhythmias has been obtained clinically [5,6], and from intact heart models [7,8]. It has been shown that sustained myocardial dilatation leads to a shortening in refractory period, and an increase in inducibility of ventricular arrhythmias [9,10]. Changes in refractory period are important in determining the minimum length of a reentry circuit for the initiation of a reentrant arrhythmia (‘wavelength’), which is the product of conduction velocity and refractory period [11].

Changes in refractoriness due to stretch vary in magnitude in different regions of the left ventricle [12], and between left and right ventricles [9]. Previous estimates of dispersion have been obtained from small numbers of sites, refractory period being estimated directly by the extrasinusus technique or indirectly from measurement of monophasic action potential (MAP) duration. However, the extrasinusus technique is both sequential and time consuming. The measurement of ventricular fibrillation intervals has been used as a means of simultaneous assessment of refractory periods from multiple sites. This technique is based on the assumption that during fibrilla-
tion, cells at an individual site will become re-excited as soon as they have recovered from the previous activation [13,14], and is supported by the demonstration of a close correlation between VF interval and effective refractory period [15]. In this study, we used this technique to assess the effect of acute left ventricular dilatation on local refractoriness.

2. Methods

2.1. Preparation

Ten adult male New Zealand White rabbits (2.8–4 kg) were studied. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Heart (NIH Publication No. 85–23, revised 1985). Rabbits were anaesthetised by IV administration of pentobarbitone (100 mg/kg) with 1000 IU heparin. The heart was rapidly excised, immediately placed in chilled saline solution and mounted onto a Langendorff perfusion apparatus. Perfusion with warm (37°C) buffer solution at a constant flow rate of 40 ml/min was achieved within one minute. The perfusate was a modified Krebs–Henseleit buffer consisting of (in mmol/l) 120.9 NaCl, 2.8 KCl, 2.5 CaCl₂, 1.5 MgSO₄, 1.5 KH₂PO₄, 24.8 NaHCO₃, 11 glucose, 2.0 sodium pyruvate, equilibrated with 95% O₂–5% CO₂ to give a pH of 7.4. The solution was pre-filtered using a 5 μm pore filter to remove contaminant particles.

Perfusion pressure was monitored continuously and ranged from 40–70 mmHg. A latex water-filled balloon was inserted in the left ventricle (LV) via the left atrium and secured with a suture. An inline membrane pressure transducer (Triton Technology, San Diego CA) monitored instantaneous isovolumetric left ventricular pressure. The balloon was inflated by means of a linear motor-driven pump. A quadrupolar pacing electrode (Cordis, Miami FL) was introduced into the right ventricle via the right atrium for induction of ventricular fibrillation.

2.2. Construction of electrode array

A silicone rubber (Sylgard) mould was made from a preserved heart, and a plaster cast made from the mould. The cast was built up and sculpted as necessary, smoothed and painted. Metal hemispheres (diameter 0.5 mm) were glued to the cast at locations corresponding to the desired electrode grid. This consisted of 8 circumferential rows of 24 electrodes spaced at 2.2 mm intervals in a rectangular grid. Within each row the inter-electrode distance varied from 2.6 mm at the base to 1.6 mm in the row closest to the apex. One additional row at the base and three rows at the apex, each comprising 12 electrodes spaced 5.2 mm to 1.4 mm, were incorporated. The cast was dipped repeatedly in air-drying liquid rubber (Trylon, Wollaston, UK) until a jacket of the desired thickness and flexibility was achieved. The hemispheres on the mould produced recesses in the latex jacket for location of the electrode sites. These recesses also prevent the electrodes from exerting undue pressure on the surface of the epicardium. The jacket was trimmed around the basal end and a slit was cut in the side corresponding to the right ventricle, from the base to within a few millimetres of the apex, to allow use with hearts of different sizes. A hole was punched in the apex to facilitate drainage. Two pairs of slit ties and four basal support threads were sewn into the jacket. Electrodes were fabricated from 0.125 mm diameter polyester-coated silver wire (Goodfellow, Cambridge, UK) by heating the end in a flame, which removed the insulation and melted the metal into a spherical terminal (diameter 400–450 μm). Wires were then threaded through the latex jacket from the inside, so that the terminals were seated in the recesses. The array is illustrated in Fig. 1. A circular perspex cradle was used to suspend the jacket over a central drain hole through which perfusate was collected, and to locate the electrode wires converging on the jacket.

2.3. Experimental protocol

The heart was lowered into the electrode array, and the tension of the jacket was adjusted with the ties to obtain signals with an amplitude of at least 20 mV peak-to-peak, while avoiding current of injury which occurred if the jacket was too tight. Typically, with the right ventricle oriented towards the slit, the electrode grid extended laterally over the entire left ventricular free wall and vertically from just below the atrio-ventricular groove to approximately 7 mm from the apex. Depending on the size of the heart, lateral columns of the array may encroach on right ventricular epicardium.

Unipolar electrograms were recorded using the aortic cannula as the indifferent electrode. Signals from each of the 240 electrodes were simultaneously amplified, filtered (0.8–550 Hz) and digitised with a resolution of 8 bits at a frequency of 1 kHz using a commercial mapping system (MapTech Inc., Maastricht, Netherlands) [16]. The data stream produced in an experiment was recorded on video tape, and played back into the computer for off-line analysis.

The heart was paced at a constant cycle length of 350 ms with a pulse width of 2 ms at twice diastolic threshold, via a pair of platinum needle electrodes in the right atrium. Following a 30 min stabilisation period, the ventricular fibrillation threshold was measured by delivery of a train of 10 constant current pulses (2 ms duration, 10 ms apart) spanning the refractory period. Current strength was increased in 5 mA steps until fibrillation was initiated and sustained for at least 4 s. This current was defined as the ventricular fibrillation threshold. Occasionally, repeated delivery of pulse trains at 100 mA were required to induce sustained VF.
Each period of VF lasted 20 min. After the first 6 min of VF, the heart was subjected to a sustained increase in pressure through balloon inflation of +20 or +40 mmHg maintained for 6 min, followed by a return to 0 mmHg. In control periods no increase in pressure was imposed. At the end of 20 min, the heart was converted to sinus rhythm by transient perfusion with 12 mmol/l K⁺ Krebs-Henseleit. Following restoration of normal rhythm, a period of 20–30 min was allowed to elapse before reinduction of VF. For each heart, at least three pressure regimes (0, +20 and +40 mmHg) were imposed. The distributions of VF intervals were obtained immediately prior to inflation (6 min, termed ‘before’), after 6 min inflated (12 min, termed ‘end’) and 8 min after the balloon was deflated (20 min, termed ‘after’) in each of the periods of VF. To determine VF inducibility in the dilated heart, the balloon was inflated during atrial pacing to produce an end diastolic pressure of 40 mmHg sustained for at least 4 min before VF threshold was again determined.

2.4. Data analysis

2.4.1. VF intervals

Recordings of 28.672 s duration (7 consecutive periods of 4096 ms) were analysed. The first stage in all analyses was the identification of activation times during fibrillation for each of the 240 channels. An automated algorithm scanned through each digitised unipolar electrogram detecting intrinsic deflections with a negative slope (−dV/dt) greater than 0.5 V/s in two or more consecutive points [15]. The point with the most negative slope was taken as the activation time, with a precision of 1 ms. Scanning for the next activation time proceeded after skipping 40 ms on the assumption that the refractory period at each site could not be less than this arbitrary limit. In practice, most inter-activation intervals were longer than 50 ms (Fig. 2). Occasional clusters of activations resulting from fractionated electrograms were thereby excluded. Visual inspection
of detected activation times indicated a very low error rate due to fractionation. At times during the recording there were brief periods when the signal was of low amplitude and the slope did not exceed the threshold. In these periods, there were gaps in activation time sequences rather than abnormally short intervals. Missed intervals produced distinct peaks in the interval histogram at multiples of the first peak. An upper threshold of VF interval was used to reject spuriously long intervals by excluding all but the first peak. The distribution of VF intervals in the first peak was sometimes skewed, so the median interval was used as an estimate of local refractoriness. Sites at which VF intervals varied greatly, for whatever reason, were excluded from further analysis: a value was assigned to a site only if the standard error of intervals was <2.5 ms [17].

2.4.2. Wavefront isolation
To estimate the frequency of activation waves, we implemented an algorithm for isolating wavefronts based on Bollacker et al. [18]. Successive and adjacent activations were considered to be part of the same wavefront. Our automated technique differed in that an activation was considered to have occurred at a single point in time at which $\frac{dV}{dt} < -0.5$ V/s rather than over a possible sequence of time points. Consequently, we have broadened our adjacency criteria to produce a better match between wavefront number obtained by the automated technique and estimates obtained by studying animated activation sequences from our experiments. Wavefront count estimates from epicardial recordings may overestimate the true number of wavefronts due to multiple sites of epicardial breakthrough of a transmural wave. Conversely, intramyocardial wavefronts which did not penetrate to the epicardium are not counted by this technique.

2.5. Statistical analysis
All data are presented as means±SE unless otherwise stated. The coefficient of variation (CV=SD×100/mean) was used as a measure of dispersion. Differences in mean VF intervals and their dispersion between experimental periods were tested with a parametric repeated measures ANOVA, and when appropriate a post test for individual differences was performed using Tukey–Kramer multiple comparisons test. A two-tailed $P$ value <0.05 was considered statistically significant.

3. Results

3.1. Ventricular fibrillation threshold
Increasing end-diastolic pressure from 0 to 40 mmHg produced a decrease in VF threshold in 9 out of 10 hearts. The mean threshold fell from 64±11 mA to 43±11 mA ($P<0.01$). Stretch resulting from this increase in pressure did not provoke any spontaneous episodes of ventricular tachycardia or fibrillation, though single premature ventricular beats were occasionally observed in some hearts.

3.2. Effect of inflation on VF intervals
Changes in VF interval over time in a single experiment are shown in Fig. 3. Mean VF interval was measured every 60 s in two separate 20 min runs. Following induction of fibrillation at 0 min, mean VF interval fell from >90 ms to about 80 ms within 3 to 4 min. The initial acceleration of fibrillation was a consistent finding. When the balloon was left uninflated at 0 mmHg, mean VF interval remained stable at this level throughout the run. Inflation of the balloon to 40 mmHg produced a fall in mean VF interval to below 60 ms. The time course of this decline was similar for all hearts studied. The mean time to half-maximal effect ($t_{1/2}$) was 1.5±0.2 min for 20 mmHg and 1.3±0.2 min for 40 mmHg inflations (not significantly different). In two preliminary experiments in which dilatation was maintained for longer periods (>10 min), there was no further change in VF interval after 5 min. The effect was reversed following deflation of the balloon to 0 mmHg. A small non-sustained overshoot was seen in 8/10 experiments.

Fig. 4 shows histograms of VF intervals in the same experiment as in Fig. 3. The effect of inflation was to decrease mean VF interval (81.3 to 60.2 ms) and to increase the dispersion of VF intervals (SD, 2.1 to 10.0 ms; CV, 2.6 to 16.7).

To exclude the possibility that the shortening in VF intervals was due to pressure between the jacket and the epicardium as the balloon was inflated in the left ventricle, two experiments were performed in which the heart was rotated 180° in the jacket from the usual orientation to obtain recordings from the epicardial surface of the right ventricle. With inflations to 40 mmHg, mean VF intervals recorded at RV sites decreased by only 3.9% (86.7 to 83.3 ms) compared with 20.4% (79.9 to 63.6 ms) at LV sites. Recordings from LV sites were also obtained without
detection limit of 0.3 mmol/l in any of the samples. The hearts were perfused with a constant flow pump. Dilatation of the left ventricle to 20 and 40 mmHg pressure produced an increase in perfusion pressure of 7.4±2.1% and 11.5±2.4% respectively.

Table 1 summarises the average effect of stretch on VF interval mean and dispersion. Inflation to 20 mmHg and 40 mmHg produced decreases in VF interval of 8% and 12%, and increases in dispersion of 48% and 64% respectively. The effect was reversible following deflation. There were no significant differences in VF interval at 0 mmHg between periods of VF in any experiment which might indicate deterioration of the heart, or a persistent effect of either VF or inflation.

3.3. Wavefront frequency

In the experimental period corresponding to Fig. 4, inflation to 40 mmHg resulted in an increase in wavefront frequency from 3119/s to 5493/s. The average effect of inflation on wavefront frequency in all experiments was a 13% increase (2600/s to 2941/s, \( P < 0.05 \)) at 20 mmHg, and a 22% increase (2478/s to 3017/s, \( P < 0.01 \)) at 40 mmHg. The effect was fully reversible.

3.4. Spatial distribution

The spatial distribution of VF intervals for the same experimental period shown in Fig. 4 is illustrated in Fig. 5. In the uninflated heart (Fig. 5A), VF intervals were very similar over the whole of the left ventricle with most of the tightening the jacket, the left ventricular epicardium lying loosely in contact with the electrode array. In this configuration, balloon inflation produced shortening of VF intervals of similar magnitude to that seen when the jacket was tightened, although the number of sites with analysable signals was reduced due to poor contact at the margins.

To determine if balloon inflation led to ischaemic production of lactate, samples of coronary effluent were collected for 1 min immediately prior to inflation, the first and last minute of inflation, and for 1 min immediately following deflation. Lactate levels did not rise above the

<table>
<thead>
<tr>
<th>Pressure (mmHg)</th>
<th>Mean (ms)±SE</th>
<th>Coefficient of variation±SE</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>End</td>
</tr>
<tr>
<td>0</td>
<td>79.4±1.9</td>
<td>78.9±1.7</td>
</tr>
<tr>
<td>20</td>
<td>78.7±1.8</td>
<td>72.4±1.9***</td>
</tr>
<tr>
<td>40</td>
<td>79.8±1.3</td>
<td>70.2±1.7***</td>
</tr>
</tbody>
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Key: *\( P < 0.05 \), **\( P < 0.01 \) for Before vs End, †\( P < 0.05 \), ††\( P < 0.01 \) for End vs After.
Fig. 5. Maps of VF intervals at 0 mmHg before inflation (A), 40 mmHg end inflation (B) and 0 mmHg after deflation (C). Each square in the rectangular arrays represents a single electrode site. Top margin is basal, the bottom apical. The median VF interval is shown colour-coded; iso-interval contours spaced at 5 ms are superimposed. Blank squares in the densely gridded area represent defective electrodes or sites excluded because of excessive noise.
Fig. 6. Maps from 10 experiments showing distribution of sites (black squares) at which VF interval decreased by more than 20 ms, following balloon inflation to 40 mmHg. The rest of the grid is shown as grey squares.
heart, but not from inflation to inflation (data not shown). Regions comprising a few sites at which VF interval lengthened were also present on most maps, and were surrounded by regions in which VF interval was unchanged or only slightly shortened.

4. Discussion

We have shown that sustained dilatation in the isolated rabbit heart produced a decrease in refractoriness, and an increase in dispersion. We also observed an increase in the apparent frequency of circulating wavefronts consistent with a decrease in wavelength (refractory time × conduction velocity). These changes would be expected to increase the propensity to develop reentrant arrhythmias, and were consistent with the observation that VF threshold decreased as a result of dilatation.

4.1. Previous studies on refractoriness

Several studies using isolated hearts under isovolumetric [8,9,12,19,20] or ejecting [10,21,22] conditions have shown a significant (7–22%) shortening in monophasic action potential duration and refractory period during a sustained increase in left ventricular volume, consistent with our findings, although Calkins [23,24] reported only minor (<2%) changes in these parameters in blood-perfused ejecting canine ventricles. Of these studies, only Reiter et al. [9] observed more frequent induction of ventricular arrhythmias with single extrastimuli after sustained dilatation. In our experiments, as in others, the increase in load required to produce these modest (10–20%) changes in refractory period was substantial: up to 2 to 3-fold increase in balloon volume, producing an rise in diastolic pressure of 20–40 mmHg. In an otherwise normal dog ventricle, a critical magnitude of dispersion of ventricular repolarisation of >90 ms was required for induction of arrhythmia by premature ventricular stimuli [25]. However, other studies show that even minor perturbations in refractoriness may be potentially arrhythmogenic if they occur in combination with nonuniformly depressed conduction [11].

To date, studies considering changes in heterogeneity of refractoriness due to dilatation have estimated dispersion from measurements at only two to six sites [9,12]. Our results have confirmed and extended these observations by showing consistent increases in dispersion of VF intervals at much greater spatial resolution (240 sites). The largest decrease in VF interval occurred in distinct regions, the location of which varied between hearts, but was frequently basal. In contrast, other workers [9,26] have found the largest decrease in refractory period after dilatation in the apical rather than the latero-basal aspect of the left ventricle. These differences may be due to variations in size and shape between hearts which we noted in our study. Differences in factors such as fibre orientation and wall thickness will contribute to differences in wall stress which could account for the heterogeneity of refractoriness in response to stretch.

4.2. Study limitations

Our interpretation of the data presented above is based on the assumption that the VF intervals measured during sustained ventricular fibrillation are correlated with local refractoriness during normal cardiac rhythms. The use of ventricular fibrillation intervals as an index of local refractoriness is based on the hypothesis that during multiple wavelet reentry [13,14], assumed to be responsible for the fibrillation, cells at any epicardial location are re-excited almost immediately following their refractory period: i.e. there is little or no excitable gap. Evidence for the lack of an excitable gap in VF has been obtained from intracellular recordings [15,27] which show no return to resting membrane potential between successive activations. This is supported by the demonstration of a close correlation between VF interval and effective refractory period [15]. However, Janse et al. [28] have recently challenged the multiple wavelet assumption, based on the observation that several single wandering spiral waves and two independent wandering reentrant wavefronts, in addition to multiple wavelets, may give rise to similar epicardial electrograms. Even if a significant excitable gap were present, refractory period will determine the maximum rate at which a region of myocardium can become reactivated. However, in the present study, we used median rather than minimum VF interval to estimate refractoriness, primarily because spurious intervals (even occurring occasionally) would confound the estimate.

Common to all epicardial mapping studies, our electrode array measured only local activation on the surface of the heart. Thus we were limited to a two-dimensional view of processes occurring in three dimensions. Epicardial measures of heterogeneity of refractoriness, important in determining the propensity for reentrant arrhythmias, therefore ignore possible endo- to epicardial differences.

We monitored for biochemical evidence of ischaemia by measurement of lactate efflux into the coronary effluent. Previous studies have shown that release of ischaemic metabolic products is most easily detected immediately after restoration of flow [29]. On this basis we would have expected lactate, if present, to have been detected in the post-deflation sample. Although the absence of lactate production in the present study would suggest that significant global ischaemia had not developed, balloon inflation might have produced compression of the endocardial surface. It is not known how sub-endocardial ischaemia would affect refractory periods measured epicardially. In any case, elevated diastolic pressures occurring in vivo may cause the same changes as balloon inflation and should not necessarily be considered as artefactual.
Acknowledgements

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References