Effect of remodelling, stretch and ischaemia on ventricular fibrillation frequency and dynamics in a heart failure model

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Abstract

Objective: The dynamics of ventricular fibrillation (VF) in the presence of heart failure (HF) are different from those in the normal heart. This has been attributed solely to HF-induced electrophysiologic remodelling. We hypothesized that acute stretch and ischaemia, which are normally present during VF, might contribute significantly to the altered VF dynamics in HF.

Methods: HF was induced in eight sheep by rapid ventricular pacing for 4–6 weeks. Eight sheep served as controls. Optical mapping of isolated hearts was performed during VF at low intraventricular pressure (0–5 mm Hg), high pressure (25–30 mm Hg, in six HF and six controls), and at low pressure after 5 min of global ischaemia (six HF, five controls). Maximum dominant frequency (DF_max), singularity point (SP) density and number of SP lasting more than one revolution (rotors) were analyzed. Possible statistical interactions between HF and ischaemia (HF/C2 ischaemia) or stretch (HF/C2 stretch) were evaluated.

Results: At low pressure, VF in HF was slower (13% reduction in DF_max) and more organized than in control: 33% less SPs and 74% less rotors with 20% longer life spans. Acute stretch did not affect DF_max but increased SP and rotors density similarly in both groups (no interaction HF/C2 stretch). In controls, ischaemia caused a marked decrease in DF_max, SP density and incidence of rotors. However, in HF animals, the ischaemia-induced decrease in SP density was virtually abolished, indicating a significant interaction HF×ischaemia (p<0.005).

Conclusions: HF remodelling decreases VF rate and increases VF organization. Acute stretch partially reverses these effects by a mechanism that is independent of remodelling. The effects of acute ischaemia on VF dynamics are significantly attenuated in HF compared to normal hearts.

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Keywords: Heart failure; Ventricular arrhythmias; Stretch; Ischaemia

1. Introduction

Heart failure (HF) is a very prevalent cardiovascular syndrome characterized by high intraventricular end-diastolic pressures and a progressive decline of cardiac contractility. HF predisposes to sudden ventricular fibrillation (VF) by mechanisms that remain unclear [1] and is also associated with higher defibrillation thresholds [2]. HF leads to downregulation of a variety of ionic currents involved in repolarization, mainly \( I_{K1} \), \( I_{Ks} \), and \( I_{to} \), which results in prolongation of action potential duration (APD) [3,4]. Experimental results indicate that repolarizing currents play a crucial role in spiral wave formation and propagation, ultimately controlling VF maintenance [5]. In addition, in failing hearts, passive electrical properties may be impaired as gap junction density is reduced [6]. Whole animal studies designed to evaluate effects of HF remodelling on VF dynamics showed a significant decrease in the number of fibrillating waves in HF animals with conflicting results regarding VF frequencies [2,7].

Although electrophysiologic changes secondary to remodelling in HF are now fairly well understood, this knowledge is still insufficient to fully explain the effects of...
HF on VF dynamics when acute factors present in fibrillating hearts are taken into account. In particular, cardiac arrest following VF causes global ischaemia and a sustained increase in intraventricular pressure [8]. These factors are known to significantly affect VF dynamics in structurally normal hearts [9,10] and may interact with HF remodelling to modulate VF dynamics. In particular, ventricular dilatation and decrease in wall thickness, as in dilated cardiomyopathy [11], may alter the way acute stretch [9] activates stretch-activated ion channels during VF, modifying accordingly fibrillation patterns. In addition, differences in response to acute ischaemia have been reported in failing vs. normal hearts [12,13].

The aim of this study was to elucidate the relative contribution of remodelling, acute stretch and acute ischaemia in VF dynamics in an ovine model of tachypacing-induced heart failure [14]. We used high-resolution optical mapping with spectral and phase analysis [15,16] to assess quantitatively wavebreak formation and rotor dynamics during VF. Experiments in isolated blood-perfused hearts [16] enabled us to study separately the effects of mechanical load and global ischaemia in both HF and control animals. Perfusion with blood was essential to avoid the rundown of VF dynamics and frequency that are frequently observed over time in isolated hearts perfused with physiological solutions [17]. Our results show for the first time that both acute sustained ventricular load and acute ischaemia significantly modulate VF dynamics in HF hearts, and that there is a specific, nonadditive interaction between effects of HF and acute ischaemia.

2. Methods

2.1. Heart failure model

The investigation conforms to 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). High-rate pacemakers (St. Jude Medical) and percutaneous right ventricular pacing leads were implanted in 11 sheep (20–25 kg). After 24 h, pacemakers in eight animals (HF group) were activated, and pacing was maintained at 220 beats/min for 4–6 weeks until clear signs of HF (tachypnea, lethargy and ascites) developed. The remaining three sheep were sham-operated. The data obtained from the latter animals were pooled together with those of five normal sheep of approximately the same weight, as the results were similar in both subsets. Transthoracic echocardiographic evaluations (Phillips Sonos 5500) were performed 10 min after deactivating the pacemaker and immediately prior to isolating the heart. Cross-sectional left ventricular (LV) thickness and internal dimensions were obtained at the level of the papillary muscles. Ejection fraction (EF) was estimated with the use of planimetry of LV end-systolic and end-diastolic areas in the cross-sectional view.

2.2. In vivo recordings

Animals were anaesthetized with pentobarbital (15 mg/kg i.v.) and maintained on isoflurane. Haemodynamic data was collected using a 7-F catheter (Berman, Arrow Int.), introduced through right external jugular vein, and a 5-F pigtail catheter (Cordis Endovascular) introduced through right carotid artery. Right atrial, right ventricular (RV), aortic and LV pressures were recorded during sinus rhythm; the maximal rate of rise in intraventricular pressure (dP/dtmax) was determined in the right ventricle (RV) and the LV. After midline sternotomy, monophasic action potentials (MAP) were recorded in four HF animals and six controls using a steerable MAP catheter (EP Technologies). MAPs were obtained sequentially from six sites on the anterior wall of both ventricles (RV: base, middle, apex; LV: base, middle, apex) at a cycle length of 400 ms. Action potential duration was measured at 90% of MAP repolarization and was averaged over six recording sites in each animal. Three minutes before the heart was isolated, VF was induced with a 9-V DC battery, and the sustained level of LV pressure during VF (VF-LVP) was determined.

2.3. Isolated heart preparation

Hearts were Langendorff perfused with a blood-Tyrode’s mixture and superfused with a warm oxygenated Tyrode’s solution as described elsewhere [16] (Fig. 1). To evaluate the effect of increased intraventricular pressure in VF dynamics, in six HF and six control hearts orifices were...
sealed, and draining tubes were connected to both ventricles via the inferior cava vein and a left pulmonary vein [18]. Both tubes were joined and connected to an open-ended cannula, the height of which controlled the level of intraventricular pressure (Fig. 1). To monitor LV pressure, an angiographic catheter (Berman, Arrow Int.) was inserted in the LV through the left atrial appendage.

2.4. Optical recordings

Five-second movies of Di-4-ANEPPS fluorescence changes were recorded at 300 frames/s using a CCD camera as described elsewhere [10]. The area of the field of view was \( -4 \times 4 \text{ cm}^2 \) (64×64 pixels) and included approximately equal portions of the anterior free walls of LV and RV (Fig. 1). Up to four movies were recorded during the same uninterrupted episode of VF. Movie 1 was recorded at low pressure (0–5 mm Hg) in all animals. In six HF and six control animals, movie 2 was recorded 3 min after increasing intraventricular pressure to the recorded in vivo values. In six HF and five control animals, movie 3 was recorded 10 min after releasing pressure and before interruption of aortic perfusion. Finally, movie 4 was recorded after 5 min of global ischaemia.

2.5. VF data analysis

Previous publications described dominant frequency mapping [19] and singularity point (SP) analysis [15,20] in detail. Maximum dominant frequency (DF\(_{\text{max}}\)) was used as an estimator of VF cycle length. Trajectories of individual SPs lasting \( \geq 10 \text{ ms} \) were tracked over 1500 consecutive frames (5 s) using a custom-made automated SP detection algorithm. A starting point of an SP trajectory was considered to be the site of a new ischaemia.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control (N=8)</th>
<th>HF (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echographic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic (cm)</td>
<td>3.7±0.7</td>
<td>4.3±0.1*</td>
</tr>
<tr>
<td>LV end-systolic (cm)</td>
<td>2.1±0.1</td>
<td>3.3±0.1*</td>
</tr>
<tr>
<td>LV end-diastolic (cm(^2))</td>
<td>10.8±0.9</td>
<td>14.7±1.1*</td>
</tr>
<tr>
<td>LV end-systolic (cm(^2))</td>
<td>3.7±0.3</td>
<td>9.1±0.6*</td>
</tr>
<tr>
<td>LV wall thickness (mm)</td>
<td>54.0±1.4</td>
<td>48.0±1.4</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>66.0±1.4</td>
<td>37.0±3.5*</td>
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<tr>
<td><strong>Haemodynamic</strong></td>
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<tr>
<td>Right atrial pressure (mm Hg)</td>
<td>5.6±1.1</td>
<td>14.7±1.7*</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>9.7±14</td>
<td>23.7±2.1*</td>
</tr>
<tr>
<td>RV (dP/dt_{\text{max}}) (mm Hg/s)</td>
<td>178±171</td>
<td>814±53*</td>
</tr>
<tr>
<td>LV (dP/dt_{\text{max}}) (mm Hg/s)</td>
<td>698±75</td>
<td>353±31*</td>
</tr>
<tr>
<td><strong>MAP</strong></td>
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<tr>
<td>Average MAP (ms)</td>
<td>248±4</td>
<td>304±17*</td>
</tr>
<tr>
<td>MAP LV (ms)</td>
<td>244±5</td>
<td>311±21*</td>
</tr>
<tr>
<td>MAP RV (ms)</td>
<td>252±7</td>
<td>298±12*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M. 
HF, heart failure; LV, left ventricular; RV, right ventricular; \(dP/dt_{\text{max}}\), maximum change in intraventricular systolic pressure over time and MAP, monophasic action potential.

\(* P<0.001,
\dagger P<0.01,
\ddagger P<0.05.\)

Fig. 2. Comparison of DF\(_{\text{max}}\), SP density, and rotor density (A, B and C, respectively) in control and HF group in the absence of ischaemia and at low intraventricular pressure (0–5 mm Hg). Values are mean±S.E.M. \(* P<0.03, \dagger P<0.02 \text{ and } \ddagger P<0.003.\) (D and E) Singularity points (SP) distribution (red dots) during a 5-s baseline recording (movie 1) in a representative control heart (D) and a failing heart (E). The rectangle shows the field of view represented in Fig. 1. There is a lower number of SP in the HF animal.
wavebreak [15,20] and its endpoint the last site of SP detection. We defined rotors as those SPs lasting more than one revolution period (defined as >1000/DF\text{max} ms).

The drifting trajectory of rotors whose initiation and termination were observed in the 5-s interval was calculated as the total sum of the frame-to-frame drifting distance. The average number of wavebreaks and rotors per square centimeter per second (SP and rotor density) were determined for each optical recording of VF. The higher the number of wavebreaks, the lower the extent of organization [15].

2.6. Statistical analysis

Results are expressed as mean±S.E.M. Unpaired t-test was used to compare echographic, haemodynamic and MAP data between normal and HF animals. Repeated-measures ANOVA were used to test individual effects of HF, ischemia and stretch in isolated hearts, as well as possible interactions between HF and ischemia and between HF and stretch. A two-tailed P value <0.05 was considered statistically significant.

3. Results

3.1. Echographic, haemodynamic and MAP findings

Rapid ventricular pacing induced significant LV dilatation and contractile dysfunction (Table 1). HF animals showed a significant increase in filling pressures and decreased dP/dt\text{max} in both ventricles. One paced animal was sacrificed after developing overt signs of congestive HF with preserved EF (58%), due to severe pacing-induced mitral regurgitation. Average MAP duration was significantly longer in HF animals than controls (304±17 ms vs. 248±4 ms, P<0.002), with no significant difference between both ventricles in any group. In vivo, 10–20 s after the onset of VF, VF-LVP reached a similar plateau level in HF and control animals (32.1±1.6 mm Hg vs. 27.4±2.4 mm Hg, respectively; NS).

3.2. Effects of HF remodelling in the absence of mechanical load or ischaemia

DF\text{max} in HF animals (8.8±0.3 Hz) was significantly lower than in controls (10.1±0.4 Hz, P<0.03; Fig. 2A). There was no significant difference in DF\text{max} between ventricles in HF (LV: 8.6±0.3 Hz vs. RV: 8.5±0.3 Hz) or in control animals (LV: 10.0±0.4 Hz vs. RV: 9.7±0.5 Hz). The density of SPs in HF animals was lower (14.1±1.8 SP/cm\textsuperscript{2}/s) than in controls (20.9±1.3 SP/cm\textsuperscript{2}/s, P<0.02), suggesting a higher degree of organization in HF (Fig. 2B). HF animals showed a 74% decrease in the density of rotors as compared to controls (0.22±0.05 rotors/cm\textsuperscript{2}/s vs. 0.84±0.2 rotors/cm\textsuperscript{2}/s; P<0.02; Fig. 2C). Rotors in HF had longer life span (167±3 ms vs. 134±5 ms; P<0.001) and larger drifting trajectories (47±3 mm vs. 39±2 mm; P<0.04) than in control.

Fig. 2D and E show the SP distribution (red dots) during a 5-s baseline recording (movie 1) in a representative control heart (D) and a failing heart (E). A lower SP density can be seen in the failing heart.

3.3. Combined effects of HF and acute mechanical load

Increasing intraventricular pressure to the level measured during VF in situ did not significantly alter DF\text{max} either in HF animals (from 8.9±0.3 to 8.9±0.4 Hz) or in controls.

Table 2
VF descriptors in baseline (movie 1) and pre-ischemic (movie 3) VF recordings

<table>
<thead>
<tr>
<th></th>
<th>Control (N=5)</th>
<th>Heart failure (N=6)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Pre-ischemic</td>
</tr>
<tr>
<td>DF\text{max} (Hz)</td>
<td>10.2±0.4</td>
<td>10.3±0.7</td>
</tr>
<tr>
<td>SP density (SP/cm\textsuperscript{2}/s)</td>
<td>21.4±1.3</td>
<td>20.9±1.8</td>
</tr>
<tr>
<td>Rotor density (rotors/cm\textsuperscript{2}/s)</td>
<td>0.96±0.3</td>
<td>0.77±0.1</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Pre-ischemic</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M. No significant differences were found comparing baseline and pre-ischemic values in any group. DF\text{max}, maximum dominant frequency.
However, stretch significantly increased SP density, from 15.5 ± 2.0 to 17.9 ± 1.9 SP/cm²/s in HF animals and from 21.3 ± 1.5 to 23.9 ± 1.1 SP/cm²/s in controls. Stretch induced an increase in rotor density, from 0.24 ± 0.06 to 0.41 ± 0.1 rotors/cm²/s in HF animals and from 0.81 ± 0.2 to 1.01 ± 0.2 rotors/cm²/s in controls. No significant statistical interaction between HF and stretch was found.

3.4. Combined effects of HF and acute global ischaemia

In the animals exposed to acute ischaemia, no significant differences in DF_max, SP density or rotor density were found between movie 1 and movie 3, taken 10 min apart prior of interruption of the perfusion (Table 2), indicating that (1) our model of VF was stable over time with regards to the parameters measured, and (2) the effects of acute stretch were fully reversible. After 5 min of ischaemia, movie 4 shows that DF_max significantly decreased in both groups, from 9.0 ± 0.3 to 6.1 ± 0.8 Hz in HF animals and from 10.3 ± 0.7 to 6.0 ± 0.5 Hz in controls (Figs. 4 and 5). However, effects of ischaemia on SP density dramatically differed in normal vs. failing hearts, as indicated by a significant interaction between HF and ischaemia (P<0.005). Five minutes of ischaemia did not modify SP density in HF hearts (from 14.7 ± 2.1 to 14.4 ± 3.3), whilst profoundly reducing it in controls (from 20.9 ± 1.8 to 9.2 ± 1.6 SP/cm²/s). Analysis of rotor density also revealed a significant interaction between HF and ischaemia (P<0.03). The number of rotors during ischaemia decreased from 0.26 ± 0.08 to 0.06 ± 0.05 rotors/cm²/s in HF animals.
and more dramatically from 0.77 ± 0.14 to 0.09 ± 0.08 rotors/cm²/s in controls, decreasing the difference observed in the absence of ischaemia.

4. Discussion

The main findings of this study are as follows. (1) Electrophysiological remodelling associated with HF modifies VF characteristics promoting organization by a reduction in the number of wavebreaks. (2) Elevation of intraventricular pressure to the level associated with VF in vivo induces the opposite effect, reducing VF organization to the same extent in HF and normal hearts. (3) The effects of ischaemia on VF complexity in HF are different from those in the normal heart.

4.1. HF remodelling and VF

Previous experimental studies in the open-chest dog showed that VF in HF is associated with a significantly lower number of wavefronts and reentrant circuits than in normal hearts [2,7]. Our data showing decrease in the density of SPs and rotors in the ovine model of HF confirm those results. Indeed, from a theoretical standpoint, since SPs flank wavefronts [15], there exists a linear relation between the density of wavefronts and SPs. Importantly, we observed for the first time that rotors are more stable in HF, as shown by their longer life span than in control.

It should be noted that previous studies on the effects of HF on VF activation frequencies in dogs yielded conflicting results. During virtual endocardial mapping, Pierpont et al. [7] showed no difference in peak frequency between HF and normal hearts. In contrast, Huang et al. [2], using an epicardial multi-electrode plaque reported a 27% reduction in activation rate in HF. Different timing of measurement, with possibly different degrees of ischaemia, may account for such a discrepancy. By preventing any possible interference due to stretch or ischaemia, we found that electrophysiological remodelling in HF induces a modest (13%) but significant reduction in DFmax. This is in good agreement with a recent human study [21] showing a modest but significant prolongation of VF cycle length in HF patients.

Lower frequencies and increased organization in the chronically failing heart may be explained on the basis of electrophysiological remodelling. HF leads to a down-regulation of repolarizing currents, including Ik1, of up to 41% uniformly across the ventricular wall [3] and prolongation of APD [4]. The CHF-specific prolongation of APD per se is most likely not relevant to VF dynamics since it is observed at slow, but not fast pacing rates [22]. We have previously shown that pharmacological blockade of Ik1 [5] decreases VF frequencies and wavebreak formation. All such effects are similar to those observed in the present study. Other experimental studies using various potassium channel blockers consistently have shown lower frequencies and increased VF organization as well [23–25]. Thus, downregulation of repolarizing potassium currents appears to be sufficient to explain a decrease in the excitation frequency and wavebreak formation during VF in failing hearts.

4.2. Effects of stretch

In previous studies, increases in ventricular volume and pressure produced significant electrophysiological changes, including abbreviation of APD [26], lower threshold for VF induction, increase in VF frequencies (up to 11–30%) and increase in complexity of the VF activation pattern [9,27]. Possibly, these changes are mediated by stretch-activated channels [28]. The increase in VF complexity in the presence of stretch was attributed either to an increase in refractory period heterogeneity [27] or to an increase in the slope of the restitution curve [9,29]. In our study, rising intraventricular pressure to in vivo levels of VF-LVP increased the number of wavebreaks and rotors to a similar extent in both groups. Unlike Chorro et al. [9], we observed no effect of stretch on VF frequencies. This discrepancy may be related to differences in techniques and species used.

Our initial expectation was that the pro-fibrillatory effects of stretch would be aggravated in failing hearts, since, according to Laplace's law, both dilatation and decrease in wall thickness [14] should cause a higher wall tension in failing as compared to normal hearts, even at the same level of intraventricular pressure. Moreover, based on the fact that the end-diastolic LV pressure is increased in HF, one may expect that the level of VF-LVP should also be higher in failing than in normal hearts. However, our results ruled out the latter assumption, as VF-LVP was essentially the same in HF and control animals. This indicates that VF-LVP is probably determined by factors other than mechanical properties of the ventricular wall. Yet although dilatation and decreased wall thickness were clearly present in the HF animals (Table 1), VF dynamics in HF hearts was not more sensitive to mechanical load than VF dynamics in normal hearts. An HF-related decrease in compliance [30] might explain this finding.

4.3. Differential effect of ischaemia in VF in HF animals

The progressive ionic and metabolic changes observed in the ventricular myocardium during the course of ischaemia have been well described [31]. Reduced excitability during ischaemia dramatically alters VF dynamics, decreasing frequency and promoting organization, mainly through a reduction in sodium conductance [10,16]. Mandapati et al. [10] observed an increase in the core size of rotating spiral waves after 5 min of global
ischaemia in the isolated rabbit heart, which they attributed to reduced excitability leading to changes in the critical curvature for propagation of rotating wave fronts. The increase in the core size was correlated to an increase in the rotation period of spiral waves. Zaitsev et al. [16] found that in porcine ischemic myocardium, a decrease in wavebreak formation paralleled the ischaemia-induced slowing of VF rate.

In the present study, global ischaemia in the isolated, but otherwise, normal ovine heart caused a decrease in DF_{max}, SP density and number of rotors during VF, which agrees well with our previous observations [10,16]. In failing hearts, however, a decrease in DF_{max} was not followed by a decrease in wavebreak formation, the latter remaining virtually unchanged by ischaemia (Fig. 4). Moreover, the significant difference (74%) in the number of rotors between HF and controls found in the absence of ischaemia was eliminated after inducing ischaemia. Overall, these results indicate that the combined effect of HF and ischaemia cannot be predicted from a linear summation of the individual effects. This phenomenon can be explained by differential effects of ischaemia in HF and normal hearts [12,13]. In particular, in failing hearts, ischaemia causes a more pronounced elevation of extracellular potassium concentration than in normal hearts [13]. It has been shown that high extracellular potassium is the major determinant of conduction abnormalities in ischemic myocardium [32]. On the other hand, elevated [K\textsuperscript{+}]\textsubscript{0} may also increase VF organization [33]. It is possible that the net effect of potassium accumulation on VF organization depends on the actual [K\textsuperscript{+}]\textsubscript{0} and on the extent to which it depresses excitability. While moderate decrease in excitability may attenuate wavebreak during VF [10,16], further decrease may revert this effect leading to so-called “slow VF” [34]. Dependence of VF organization of ischaemia, elevated [K\textsuperscript{+}]\textsubscript{0} and reduced excitability have been explained in terms of their effects on the core size of the spiral wave [10], APD restitution [33], conduction velocity restitution [34] or stability of the filament of a three-dimensional scroll wave [35]. Clearly, more experimental and theoretical work is required to better understand the fundamental relationship between excitability and VF organization.

Failing hearts typically show impaired calcium handling, with a slower decay in L-type calcium current, contributing to longer APD in sinus rhythm and a net increase in intracellular calcium [4]. Intracellular calcium concentration affects the function of several ion channels and transporters and alters the resistance between cells through gap junctional conductance [4]. Both ischaemia and VF promote intracellular calcium overload [12,36]. As changes in intracellular calcium concentration [37] and L-type calcium current influence VF dynamics [38], any difference in calcium handling between HF and normal animals during ischaemia may also contribute to the differential effects of global ischaemia on VF organization in normal and HF hearts.

4.4. Study limitations

The role that fibrosis may play in VF dynamics in human HF is not addressed in this paper due to lack of fibrosis in the present model. Due to the limited field of view in large animals, our conclusions are restricted to a fraction of the total number of epicardial activation patterns that may be seen during VF. However, a similar extent of HF remodelling has been described at the epicardial, midmyocardial and endocardial layers [3].

In our study of the effects of ischemia, we did not fully eliminate oxygen in the superfusate, which could theoretically affect the dynamics of VF, as measured by optical recordings from the epicardium. Uninterrupted maintenance of superfusion throughout the ischemic episode was essential for reliable temperature control in our experimental setup. It should be noted, however, that the resulting subepicardial layer with incomplete ischemia is at most 500–600 μm thick [39]. Since this is less than the spatial extent of the upstroke of a propagating action potential (~1 mm), it is very unlikely that the subepicardium exhibited wave dynamics that were distinct from those in the deeper, fully ischemic layers. Even in the unlikely event that the wave dynamics in the subepicardium were different, about 50% of the epifluorescence was collected from depths as large as 0.5–2.0 mm [40], which included fully ischemic tissue. Thus, regardless of whether or not ischemic VF dynamics were fully represented by the recorded epifluorescent signals, the statistically significant difference between normal and CHF hearts supports the validity of our results.

The natural evolution of activation patterns during VF in structurally normal but globally ischaemic hearts is not monotonic, but rather bi- or triphasic [41]. To the best of our knowledge, the time course of VF organization during global ischaemia in failing hearts was not studied in detail. Thus, it remains uncertain whether the observed changes in VF organization in failing hearts at the fifth minute of ischaemia are the result of a modification in the rate or the magnitude of the ischaemia-induced alterations, or both. Nevertheless, our conclusion about specific interaction between the effects of HF and ischaemia on VF dynamics remains statistically valid regardless of possible changes in the time course. However, it is obvious that further studies are warranted to address this important issue.

4.5. Possible clinical implications

This study provides the first demonstration that VF organization in failing hearts is significantly modulated by the acute effects of both sustained intraventricular pressure and ischaemia. The results support the contention that the effects of VF-associated ischaemia in failing hearts cannot be inferred directly from observations of the effects of ischaemia and high intraventricular pressure in normal hearts. Therefore, any pharmacological study on VF related to HF in patients or animal models should properly account for
possible complex interactions between the drug under study and the presence of ischaemia and/or HF.

Based on our results, it is reasonable to speculate that in the clinical setting, the interactions between HF and ischaemia might modify the response to any given therapeutic approach to terminate VF. However, we should note that the relevance of VF organization to defibrillation outcome in the combined setting of HF and global ischaemia is well beyond the scope of this study and remains uncertain. No significant correlation between VF rate and defibrillation threshold was found in a recent study in HF patients [21]. Our finding that, in the presence of HF, ischaemia does not change wavebreak density (see Fig. 4B) suggests that in failing hearts, unlike normal hearts, the defibrillation efficacy should not deteriorate that much with time elapsed after VF onset. Yet further studies are required to determine the true value of this logical proposition.

Obviously, the present study does not imply any immediate clinical change on VF treatment in HF. However, it shows how factors modulating cardiac ion channels, including HF remodelling, stretch and ischaemia, profoundly modify fibrillation dynamics. These findings support the idea that fibrillation maintenance might be vulnerable to therapeutic interventions on ion channels [5]. Such interventions will have to take into account the HF-specific fibrillation dynamics, as they will be present in the majority of patients prone to suddenly develop VF.

Acknowledgments

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References

[34] Wu TJ, Lin SF, Weiss JN, Ting CT, Chen PS. Two types of ventricular fibrillation in isolated rabbit hearts: importance of excitability and action potential duration restitution. Circulation 2002;106.