Pathophysiological mechanisms of arrhythmogenic right ventricular disorders
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CHAPTER 8

Ventricular fibrillation hampers the restoration of creatine-phosphate levels during simulated cardiopulmonary resuscitations

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Abstract

Aims Recurrences of ventricular fibrillation (VF) during cardiopulmonary resuscitation (CPR) are associated with a reduced chance of survival. The effect of VF during CPR on the myocardium is unknown. We tested the hypothesis that VF during simulated CPR reduces the restoration of the myocardial energy state and contractile function.

Methods and results Twelve porcine hearts were isolated and perfused with the pig’s own blood. First, cardiac oxygen consumption was measured by blood gas analysis. Secondly, we simulated sudden cardiac arrest by VF (7 min VF, zero flow) followed by simulated CPR (7 min, 0.3 mL/g/min perfusion rate) in the absence and presence of VF [six hearts were maintained in VF (VF-group), six were defibrillated (defib-group)]. The VF increased the cardiac oxygen consumption by 71% (0.87 ± 0.12 vs. 1.49 ± 0.14 μmol O2/g/min; mean ± SEM, P< 0.001) compared with a ventricular rhythm of 62 beats/min. The presence of VF during simulated CPR after 7 min of cardiac arrest hampered restoration of myocardial creatine-phosphate levels compared with defibrillated hearts (61 ± 9 vs. 87 ± 7% of baseline values, respectively; P< 0.05). The cardiac contractile function was significantly higher in the defib- than in the VF-group (area under the pressure curve 2.29 ± 0.22 vs. 1.72 ± 0.14 s×mm Hg respectively; P< 0.05).

Conclusions These data demonstrate that the cardiac oxygen consumption is increased by VF and that the presence of VF during CPR hampers the restoration of the myocardial energy state and contractility. Strategies that reduce VF duration without disrupting chest compressions will benefit the restoration of the cardiac energy state during resuscitations.
Introduction

Prompt initiation of cardiopulmonary resuscitation (CPR) and early defibrillation are major determinants of the survival of sudden cardiac arrest by ventricular fibrillation (VF).1–3 Countershocks have a high defibrillation success rate but recurrences of VF during CPR are common.4–6 Experimental studies have demonstrated that interruption of chest compressions during resuscitations for detection and defibrillation of recurrent VF worsens the resuscitation outcome.7,8 The 2005 resuscitation guidelines (GL2005) have incorporated these findings and aimed to minimize the interruption of chest compressions by promoting immediate resumption of chest compressions after defibrillation attempts (without confirmation of the success of a defibrillation shock) and by prolongation of each CPR cycle after each rhythm assessment from 1 to 2 min. As defibrillation success is <100% and recurrent VF is observed in at least 60% of cases, fewer interruptions of chest compressions for rhythm assessment and defibrillation may result in more and longer episodes of VF. Indeed, we have shown that under GL2005 patients spend 48% more time in VF as under the 2000 resuscitation guidelines.6 Ventricular fibrillation involves a high-frequency of activation and contraction of the myocardium and is associated with an increased cardiac oxygen consumption in non-ischaemic conditions.9 In the setting of cardiac arrest, such conditions will only be present briefly because the onset of VF is rapidly followed by deterioration of cardiac perfusion.8 The effect of VF on the myocardial energy status during ischaemic conditions encountered thereafter is unknown. However, several observations have indicated that VF may directly influence the chance of survival of cardiac arrest: recurrences of VF during CPR are inversely related with survival from out-of-hospital cardiac arrest (OHCA).10 This inverse relation with survival also exists with ‘VF burden’, the cumulative time a patient is in recurrent VF.6

In this study, we tested the hypothesis that the increased cardiac energy consumption by VF during CPR reduces the restoration of the cardiac energy state and contractility. We therefore determined the restoration of the creatine-phosphate (CrP) levels and contractility during simulated resuscitations in the isolated, Langendorff-perfused porcine heart.

Methods

The experimental protocol complied with the guide for the Care and use of Laboratory Animals published by the US National Institute of Health and was approved by the institutional animal experiments committee.

Experimental set-up

Twelve male pigs (28.9 ± 2.6 kg; mean ± SD) were premedicated with an intramuscular injection of ketamine (10–15 mg/kg, Animal Health BV, Bladel, The Nether-
lands) and midazolam (1.0 mg/kg, F. Hoffmann – La Roche Ltd., Basel, Switzerland). After induction of anaesthesia with pentobarbital (15–20 mg/kg IV, CEVA Santé Animale, La Ballastière, France), the animals were intubated and ventilated with a mixture of ambient air and oxygen. The thorax was opened by median sternotomy, heparin (Leo Pharma BV, Breda, The Netherlands) was infused, and blood was collected from the superior caval vein. Ventricular fibrillation was induced by a direct current to prevent air from entering the coronary vasculature upon removal of the heart. The hearts were rapidly removed, submerged in ice-cold modified Tyrode’s solution and weighed.

The aorta was cannulated, connected to a Langendorff set-up and defibrillated. The heart was perfused with the collected blood, which was diluted to a haematocrit of 0.30 vol./vol. with modified Tyrode’s solution (NaCl 128.0 mmol/L, KCl 4.7 mmol/L, CaCl₂ 1.5 mmol/L, MgCl₂ 0.7 mmol/L, NaHCO₃ 28.0 mmol/L, NaH₂PO₄ 0.5 mmol/L, glucose 11.0 mmol/L, insulin 10 IU/L, heparin 5000 IU/L) and was gassed with O₂/CO₂ 95/5%. The perfusion rate was normalized to heart weight and continuously measured using an ultrasonic flow sensor (Transonic Systems Inc., Ithaca, NY, USA). Intramural myocardial temperature was monitored throughout the experiment.

Total AV block was created by clamping the AV node. A bipolar hook electrode was positioned at the basal left ventricular free wall. A compliant latex balloon (length 4.2 cm, diameter 2.2 cm) was inserted in the conical part of the right ventricular outflow tract (RVOT) and secured to the pulmonary artery to record pressure curves throughout the experiment. The RVOT was selected because of its conical shape and because it reduced the chance of puncturing of the balloon by transmural sampling of the apical left ventricular myocardium as described in the experimental protocol. The balloon was filled with warmed modified Tyrode’s solution until a diastolic pressure of 9–10 mm Hg was reached. The pressure in the balloon was monitored using a Gould-Statham P23 ID pressure transducer (Gould Instruments, Oxnard, CA, USA). Right ventricular outflow tract pressure curves were continuously recorded on paper using a Graphtec WR5000 Thermal Array Recorder (Graphtec Corp., Yokohama, Japan) at a speed of 1 mm/s; selected episodes were recorded at 25 mm/s. A sheet of aluminium foil was positioned posterior of the heart for thermal isolation. The heart was allowed to equilibrate for 10 min before the start of the experimental protocol.

**Experimental protocol**

Cardiac oxygen consumption during ventricular fibrillation

Before simulation of CPR for sudden cardiac arrest by VF, we determined the effect of VF on the oxygen consumption during non-ischaemic conditions using blood gas analysis. Blood samples were obtained from the aorta (arterial) and the coronary sinus (venous) using glass syringes during a constant perfusion rate of 1.1 mL/g/min under two conditions: (i) spontaneous ventricular rhythm and (ii) 1 min after the
initiation of VF by a direct current. Blood gas analysis and haemoglobin measurements were performed as soon as possible (within 5 min) after sampling using a Radiometer ABL505 blood gas analyser (Radiometer Medical ApS, Brønshøj, Denmark). After obtaining the blood samples, the hearts were defibrillated and allowed to equilibrate for 15 min before continuation of the protocol. Using the blood gas data, we calculated the O₂ content (CaO₂) and the cardiac O₂ consumption as follows:

\[
\text{CaO}_2 (\text{ml O}_2 / \text{dl blood}) = \text{Hb(g/dl)} \times \text{SaO}_2 \times 1.36 + \text{PaO}_2 (\text{mmHg}) \times 0.0031
\]

where SaO₂ denotes oxygen saturation and PaO₂ partial oxygen tension.

\[
\text{Cardiac O}_2 \text{ consumption(μmol/g/min)} = \frac{(\text{Arterial CaO}_2 - \text{Venous CaO}_2) \times \text{blood flow(dl/min)} : \text{heart weight(g)}}{22.4 \times 10^3}
\]

The CO₂ content (TCO₂) was calculated according to

\[
\text{TCO}_2 (\text{mmol/l}) = [\text{HCO}_3^-](\text{mmol/l}) + 0.03 \times \text{PaCO}_2 (\text{mmHg})
\]

and the cardiac CO₂ production according to

\[
\text{Cardiac CO}_2 \text{ production(μmol/g/min)} = (\text{Venous TCO}_2 - \text{Arterial TCO}_2) \times \frac{\text{blood flow(l/min)} \times 10^3 : \text{heart weight(g)}}{	ext{22.4}}
\]

Simulated cardiopulmonary resuscitation
Figure 1 shows a diagrammatic representation of the experimental protocol of the simulated CPR for sudden cardiac arrest by VF. At baseline, the heart was stimulated at twice the diastolic threshold from the basal left ventricular free wall at a cycle length of 400 ms during perfusion at 1.1 mL/g/min. A transmural myocardial sample was obtained from the apical left ventricle while carefully avoiding the local vasculature using a hollow rotating drill (diameter 4 mm) and snap frozen in liquid nitrogen. Pressure curves from the RVOT were recorded during stimulation. Then, sudden cardiac arrest by VF was simulated by initiation of VF using a direct current and stopping of the perfusion pump during 7 min. This period was chosen to reflect the average arrival time of paramedics to the scene of OHCA in the Netherlands. During these 7 min, the intramyocardial temperature was maintained using two heating lamps aimed at the heart. Intramyocardial temperature was continuously monitored with a miniature thermistor incorporated in a needle. After 7 min, a second, left ventricular transmural myocardial sample was obtained and snap frozen.
After 7 min of global ischaemia and VF, CPR was simulated by low flow perfusion at 0.3 mL/g/min. Two groups were studied: (i) hearts that were defibrillated using a 25 J shock ($n=6$, defib-group), (ii) hearts that were not defibrillated ($n=6$, VF-group). In each group, a transmural myocardial sample was obtained and snap frozen at 8, 10, and 14 min (see Figure 1). If myocardial sampling reinitiated VF in the defibrillation group, the hearts were defibrillated as soon as possible. After 14 min, the perfusion rate was normalized to 1.1 mL/g/min in both groups, and the hearts of the VF-group were defibrillated. All hearts were then paced from the basal left ventricular free wall at a cycle length of 400 ms as before. Pressure curves from the RVOT were recorded 15, 20, 30, 45, and 60 min after the start of the simulated cardiac arrest. One heart in the defib-group developed a small, well-defined cyanotic area on the RVOT following restart of normal perfusion and was excluded from analysis of the RVOT pressure curves. End diastolic pressure and the systolic area under the curve were calculated from five consecutive scanned pressure curves with the use of Adobe Photoshop CS2 (Adobe Systems Integrated, San Jose, CA, USA).

**Biochemical analysis**

During ischaemia, the decrease in ATP levels themselves is limited during the first ~15 min. However, the free energy available by ATP hydrolysis is reduced rapidly and is reflected by the CrP concentration. We therefore chose to determine the CrP concentration as a marker of the myocardial energy state. The tissue samples were freeze-dried overnight, and epi- and endocardial myocardium was removed. The remaining midmural myocardium was pulverized, weighed and deproteinized in 4% perchloric acid. The supernatant was neutralized to pH 7.0 with triethanolamine/KCl buffer. The amount of CrP in the neutralized extracts was determined by the direct enzymatic method of Lowry and Passonneau.
Increased cardiac energy consumption during VF

Statistical analysis
Data are presented as mean ± SEM unless indicated otherwise. Normally distributed biochemical data, intramyocardial temperature, and perfusion rates were compared between groups using one-way analysis of variance (ANOVA). Factor correction was applied on the RVOT pressure curves of the defib- and VF-group based on the pressure curves prior to the start of the simulated resuscitations.15 End-diastolic pressure and systolic area under the curve of the corrected pressure curves were compared between groups using one-way ANOVA. The level of significance was set at P< 0.05.

Results

Cardiac oxygen consumption during ventricular fibrillation
Using blood gas analysis, we confirmed9 that VF increases the cardiac oxygen consumption: VF increased both the cardiac oxygen consumption (1.49 ± 0.14 vs. 0.87 ± 0.12 μmol/g/min; mean ± SEM, P< 0.001, paired t-test) and CO₂ production (1.18 ± 0.12 vs. 0.71 ± 0.13 μmol/g/min; mean ± SEM, P< 0.001, paired t-test) compared with a spontaneous ventricular rhythm of 62 beats/min. In accordance, VF induced significant changes in the venous pH, pCO₂, HCO₃⁻, and base excess (Table 1).

Table 1. Blood gas data during spontaneous rhythm and ventricular fibrillation

<table>
<thead>
<tr>
<th></th>
<th>Arterial</th>
<th>Venous</th>
<th>Arterial</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>62 ± 5</td>
<td>NA</td>
<td>1.09 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Perfusion rate (mL/g/min)</td>
<td>1.10 ± 0.00</td>
<td>1.09 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.1 ± 0.1</td>
<td>38.1 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.415 ± 0.008</td>
<td>7.392 ± 0.007</td>
<td>7.413 ± 0.006</td>
<td>7.386 ± 0.007*</td>
</tr>
<tr>
<td>pCO₂ (mm Hg)</td>
<td>41.9 ± 0.3</td>
<td>45.2 ± 0.4</td>
<td>41.6 ± 0.4</td>
<td>46.5 ± 0.4*</td>
</tr>
<tr>
<td>pO₂ (mm Hg)</td>
<td>646.9 ± 7.7</td>
<td>130.5 ± 15.1</td>
<td>636.2 ± 10.4</td>
<td>76.0 ± 4.0*</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>26.4 ± 0.5</td>
<td>26.9 ± 0.4</td>
<td>26.3 ± 0.5</td>
<td>27.3 ± 0.5*</td>
</tr>
<tr>
<td>Base excess (mmol/L)</td>
<td>2.0 ± 0.6</td>
<td>2.2 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>2.4 ± 0.5*</td>
</tr>
<tr>
<td>sO₂</td>
<td>97.4 ± 0.6</td>
<td>96.4 ± 1.4</td>
<td>99.8 ± 0.0</td>
<td>90.2 ± 2.3*</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>9.8 ± 0.1</td>
<td>9.8 ± 0.1</td>
<td>9.7 ± 0.1</td>
<td>9.7 ± 0.1</td>
</tr>
<tr>
<td>O₂ consumption (μmol/g/min)</td>
<td>0.87 ± 0.12</td>
<td>1.49 ± 0.14*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂ production (μmol/g/min)</td>
<td>0.71 ± 0.13</td>
<td>1.18 ± 0.12*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±SEM, NA, not applicable. *P < 0.001 VF vs. spontaneous rhythm, paired t-test.

Simulated cardiopulmonary resuscitation
The CrP level decreased equally during 7 min of VF without cardiac perfusion in the defib- and VF-group (baseline 52.0 ± 2.7 vs. 51.8 ± 3.6 μmol/g dry wt., 7 min. of VF without cardiac perfusion 3.5 ± 0.3 vs. 4.1 ± 0.7 μmol/g dry wt.; defib- vs. VF-group, respectively; P > 0.05 between groups). Restoration of CrP during simulated CPR was significantly higher in the defib- than in the VF-group (Figure 2). After 7 min of simulated CPR following 7 min of simulated cardiac arrest, the CrP levels reached
87 ± 7% of baseline levels in defib-group vs. 61 ± 9% in the VF-group ($P<0.05$). Moreover, CrP had reached a plateau in the VF-group, whereas it was still increasing in the defib-group (Figure 2).

![Graph showing myocardial creatine-phosphate (CrP) normalized to baseline levels during simulated cardiopulmonary resuscitation.](image)

**Figure 2** Myocardial creatine-phosphate (CrP) normalized to baseline levels during simulated cardiopulmonary resuscitation. Creatine-phosphate restoration was significantly higher in the defib-group (open symbols) than in the VF-group (closed symbols) after 7 min of simulated cardiopulmonary resuscitation.

The perfusion rate and average temperature during the simulated CPR did not differ significantly between groups (0.304 ± 0.005 vs. 0.300 ± 0.004 mL/g/min and 36.9 ± 0.2 vs. 37.2 ± 0.1°C; defib- vs. VF-group, respectively; $P>0.05$). Transmural myocardial sampling during simulated CPR always initiated VF in the defib-group. The average number of defibrillation shocks in the defib-group was 5.3 (range 4–8). In the VF-group, each heart received only one shock during simulated CPR. Hearts in the defibrillation group spent $38 \pm 9$ s in VF during the 7 min of CPR.

No significant differences were observed in the diastolic pressure between the groups at any moment. The systolic area under the curve increased in five out of six hearts in the VF-group 1 min after defibrillation. However, this increase was followed by a decline in pressure curves (see Figure 3). At 60 min after the start of simulated CPR, the area under the curve was significantly lower in the VF- than in the defib-group (Figure 3).
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Figure 3. Typical example of a right ventricular outflow tract pressure curve of a heart in the VF-group and graph showing the area under the curve in the VF- and defib-groups. The peak pressure and area under the curve increased after defibrillation in five out of six VF hearts but was followed by a marked decline of the pressure curves. At 60 min the AUC was significantly lower in the VF- than in the defib-group.
Discussion

In this study, we confirmed that VF markedly increases the cardiac oxygen consumption during non-ischaemic conditions that are present briefly after the onset of sudden cardiac arrest by VF. Furthermore, we demonstrated that VF during simulated CPR after a prolonged episode of untreated VF hampers the restoration of the myocardial energy state and of the ventricular contractile function.

In the setting of OHCA by VF, defibrillation is usually delayed for ~9 min. Our observations demonstrate that in this time frame the myocardial energy state will have deteriorated markedly, unless efficient CPR is delivered. However, in many cases of OHCA the myocardial function cannot be sufficiently restored even when optimal CPR is performed and the patient dies either in asystole or with a non-perfusing rhythm. Hence, any factor that adversely affects the restoration of myocardial energy state during CPR should be removed. Our findings suggest that continued or recurrent VF is an important adverse factor and that immediate termination of (recurrent) VF without disruption of chest compressions may benefit the outcome of resuscitations.

The GL2005 guidelines involved reduction of the number of interruptions of CPR by immediate resumptions of chest compressions after the delivery of a defibrillation shock (without confirmation of the success of a defibrillation shock) and the prolongation of each CPR cycle from 1 to 2 min. Our results may in part explain why implementation of the GL2005 did not meet expectations in terms of patient outcome with several studies failing to demonstrate increased survival, as they may have led to improved coronary perfusion but at the same time have increased the duration of VF and cardiac energy consumption during CPR. The new 2010 resuscitation guidelines have made no essential change to this approach.

Recently, new techniques have emerged that may enable rapid detection and defibrillation of recurrent VF without interruption of chest compressions. First, advanced electrocardiographic filtering techniques have been shown to remove motion artefacts from the electrocardiogram during chest compressions. Secondly, chest compression devices or the wearing of protective gloves may enable defibrillation of VF during continuous chest compressions. These developments may reduce the need for ‘hands-off” periods for defibrillation and rhythm assessment during CPR and may enable an ideal resuscitation strategy with minimal interruptions of CPR and a minimal VF burden.

Methodological considerations

In this study, we used isolated, Langendorff-perfused hearts because it enabled us to control the coronary perfusion rate for the determination of the cardiac oxygen consumption and it allowed us to obtain transmural myocardial samples. Transmural sampling is essential for the determination of the myocardial energy state because
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diffusion of oxygen spares the subendo- and subepicardium from the most severe ischaemia. In contrast to the \textit{in vivo} heart, the isolated, Langendorff-perfused heart contracts in an unloaded state, in the absence of circulating cathecholamines and is denervated. \textit{In vivo}, defibrillation of prolonged VF is usually followed by a non-organized and most likely non-perfusing rhythm. Similar to our model, the loading conditions \textit{in vivo} are independent of a recurrence of VF. Circulating cathecholamines and innervation are unlikely to annul the increased myocardial energy consumption by VF because it is caused by the intrinsic high frequency of activation and contraction of VF. However, the extent by which VF increases the energy consumption may be affected, which is likely larger in the loaded than in unloaded state. Our data may therefore represent an underestimation of the effects \textit{in vivo}.

We observed that cardiac contractility transiently increased during several minutes after reperfusion in the VF group. To the best of our knowledge, this has not been described before in the setting of resuscitation. We speculate that the combination of ischaemia/reperfusion and rapid activation by VF caused calcium overload, leading to a temporary increase in contractility.

In our study left ventricular transmural samples showed an almost complete return to baseline levels of CrP, whereas the RVOT pressures had not returned to baseline, but were still declining, albeit slower in the defib- than in the VF-group. This indicates that there is no direct 1 : 1 correlation between CrP alone and RVOT contractility.

\textbf{Conclusions}

Ventricular fibrillation increases cardiac oxygen consumption and hampers restoration of the myocardial energy state and ventricular contractile function during simulated resuscitations following 7 min of cardiac arrest. Early detection and defibrillation of recurrent VF during CPR without interruption of chest compressions may improve survival of OHCA by reduction of the energy consumption by the ischaemic myocardium.

\textbf{Acknowledgements}

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References


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