Heart failure secondary to chronic pulmonary arterial hypertension : cardiac imaging and electrophysiologic characteristics
Hardziyenka, Maxim

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Right ventricular failure secondary to pressure overload is not associated with ventricular arrhythmias because wavelength is prolonged

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Submitted
Right ventricular failure secondary to pressure overload is not associated with ventricular arrhythmias because wavelength is prolonged

List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>CTEPH</td>
<td>chronic thromboembolic pulmonary hypertension</td>
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<tr>
<td>ERP</td>
<td>effective refractory period</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricle</td>
</tr>
<tr>
<td>MCT</td>
<td>monocrotaline</td>
</tr>
<tr>
<td>NYHA</td>
<td>New-York Heart Association</td>
</tr>
<tr>
<td>RV</td>
<td>right ventricle</td>
</tr>
<tr>
<td>VF</td>
<td>ventricular fibrillation</td>
</tr>
<tr>
<td>VT</td>
<td>ventricular tachycardia</td>
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</tbody>
</table>
Abstract

**Rationale:** Although right ventricular (RV) failure (RVF) due to pressure overload is associated with structural and electrophysiologic RV remodeling, the incidence of ventricular tachyarrhythmias is unknown in patients with chronic thromboembolic pulmonary hypertension (CTEPH), while it is low in other types of pulmonary hypertension.

**Objective:** We aimed to assess the incidence of ventricular tachyarrhythmias in patients with CTEPH. Moreover, we aimed to relate our findings with structural and functional properties of the remodeled RV and left ventricle (LV) by studying the monocrotaline (MCT) rat model in which RVF is mediated by pulmonary hypertension.

**Methods and Results:** We studied 122 consecutive CTEPH patients with 12-lead ECG recording, pulmonary angiography, and RV catheterization. In 55 patients, cardiopulmonary exercise was conducted to assess the incidence of arrhythmias. Hearts of RVF and control rats were used to conduct epicardial mapping and cellular electrophysiologic studies; RV and LV tissues were characterized by Picro-Sirius Red staining, Western blotting, and immunohistochemistry. In CTEPH patients, no ventricular tachyarrhythmias were documented, neither at rest, nor during exercise testing. Only single supraventricular and ventricular extrasystoles occurred during exercise testing in 11 (20%) and 13 (24%) patients, respectively. Hearts of RVF rats exhibited no increased susceptibility to ventricular tachyarrhythmias; this was associated with increased excitation wavelength in both ventricles.

**Conclusions:** The incidence of ventricular tachyarrhythmias in patients with severe CTEPH is low. In parallel, susceptibility to ventricular tachyarrhythmias of MCT rats in RVF is low. This is associated with increased excitation wavelength in both ventricles, which protects the heart against the occurrence of re-entrant excitation.

Key words:
right ventricular failure - pulmonary hypertension – arrhythmia - electrophysiology
Right ventricular failure secondary to pressure overload is not associated with ventricular arrhythmias because wavelength is prolonged

Introduction
Life-threatening ventricular tachyarrhythmias (ventricular tachycardia/fibrillation, VT/VF) are frequently observed in patients with left ventricular (LV) heart failure. The pathophysiologic basis of this increased VT/VF incidence has been largely elucidated by studies which have addressed electrophysiologic and structural remodeling of LV during LV failure. In contrast, little is known about VT/VF incidence and electrophysiologic/structural remodeling of right ventricle (RV) during RV failure (RVF). Yet, RVF also poses a major public health problem. Chronic thromboembolic pulmonary hypertension (CTEPH) leads to RV pressure overload and may culminate in RVF with associated high mortality rates. However, it is unknown whether CTEPH patients have an increased incidence of VT/VF. In patients with primary pulmonary hypertension and in experimental animal models of RVF, RV pressure overload was not accompanied by increased VT/VF incidence.

Sustained VT/VF is often based on reentrant excitation, which critically depends on reduction in the wavelength of the excitation wavefront (the mathematical product of conduction velocity and effective refractory period, ERP). Experimental and clinical studies have indicated that RVF is associated with electrophysiologic and structural remodeling, similar to changes in LV during the development of LV failure. Prolongation of action potential (AP) duration in RV myocytes is a consistent finding in these studies. Recently, we demonstrated that RV ERP is prolonged in CTEPH patients. This might increase excitation wavelength, thereby rendering the heart less susceptible to reentrant excitation. To test the hypothesis that RVF secondary to pressure overload is not associated with increased VT/VF risk, we assessed the incidence of VT/VF in CTEPH patients. To probe VT/VF vulnerability and its determinants, we studied structural and functional properties of ventricular myocardium in a rat model of RV pressure overload and RVF induced by monocrotaline (MCT).
Materials and Methods

Patients

We retrospectively studied 122 consecutive CTEPH patients (mean age 55±2 years, 71 women) who were referred to the Academic Medical Center. CTEPH was diagnosed as reported previously \(^1\). Medical histories of all patients were explored. All patients underwent 12-lead ECG recording, pulmonary angiography, and RV catheterization as part of the routine preoperative work-up. In 55 patients ECG was also recorded during maximal cardiopulmonary exercise testing (bicycle stress test). Plasma brain natriuretic peptide levels \(^2\) and 6-minute walking distance \(^3\) were determined in 96 and 108 patients, respectively. Investigations were approved by the local institutional review board.

Table 1: Characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six-minute walking distance, m</td>
<td>426±6 (n=108)</td>
</tr>
<tr>
<td>NYHA class, I/II/III/IV</td>
<td>0/25/86/11</td>
</tr>
<tr>
<td>Plasma brain natriuretic peptide level, pmol/l</td>
<td>11.8 (0.7-189.9)(^a) (n=96)</td>
</tr>
<tr>
<td>Systolic arterial pressure, mmHg</td>
<td>129±21 (n=112)</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mmHg</td>
<td>77±14 (n=112)</td>
</tr>
<tr>
<td><strong>Electrocardiography at rest</strong></td>
<td></td>
</tr>
<tr>
<td>PQ duration, ms</td>
<td>164±2</td>
</tr>
<tr>
<td>QRS duration, ms</td>
<td>97±2</td>
</tr>
<tr>
<td>QTc duration, ms</td>
<td>428±1</td>
</tr>
<tr>
<td>Supraventricular tachycardia, n (%)</td>
<td>9 (7)</td>
</tr>
<tr>
<td>First-degree AV block, n (%)</td>
<td>8 (7)</td>
</tr>
<tr>
<td>RBBB, n (%)</td>
<td>9 (7)</td>
</tr>
<tr>
<td>First-degree AV block combined with RBBB, n (%)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>LBBB, n (%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>Catheterization</strong></td>
<td></td>
</tr>
<tr>
<td>Mean pulmonary arterial pressure, mmHg</td>
<td>43±2</td>
</tr>
<tr>
<td>Total pulmonary resistance, dynes/sec/cm(^{-5})</td>
<td>811±16 (n=114)</td>
</tr>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>9.0±1.7 (n=110)</td>
</tr>
<tr>
<td>Cardiac index, L/min/m(^2)</td>
<td>2.5±0.5 (n=114)</td>
</tr>
</tbody>
</table>

Characteristics were analyzed in 122 patients, unless otherwise indicated. Data are mean±SEM or \(^a\)median (interquartile range).
Animal model

The study was approved by the institutional animal use committee. Animals were cared for in accordance with institutional guidelines for the care and use of laboratory animals. Twenty seven week-old male Wistar rats (obtained from Harlan, The Netherlands) (225-285g) received an intraperitoneal injection of MCT (60mg/kg) to induce pulmonary hypertension and, secondary, RV hypertrophy and RVF. Eighteen control animals were injected intraperitoneally with the same volume of saline (3ml/kg). Transthoracic echocardiography was performed serially under 3% isoflurane inhalation anesthesia. RVF was defined by clinical parameters. The physical state of the animals was monitored daily. Animals were sacrificed as soon as they developed RVF (RVF rats). Control rats were sacrificed when they had reached the same age as RVF rats. The rats were euthanized by intraperitoneal injection of 100mg/kg pentobarbital, preceded by 0.5ml (2500U) heparin. The chest was opened and the presence of pleural effusion was assessed, the aorta was cannulated, and the heart was dissected and transferred to a Langendorff perfusion set-up. The lungs and liver were dissected, blotted dry and weighed. Epicardial mapping, cell dimensions, cellular electrophysiology, and histology were performed as described below. The whole heart was weighed, and the weights of RV and LV (including interventricular septum) were assessed separately after epicardial mapping experiments.

Epicardial mapping and data analysis

Hearts were perfused according to the Langendorff technique at constant pressure (70cm H_2O) with Tyrode’s solution (37°C) containing (mM): NaCl 130, KCl 5.6, CaCl_2 2.2, MgCl_2 0.55, NaHCO_3 24.2, and glucose 11.1 equilibrated with 95% O_2 and 5% CO_2 (pH=7.4). Extracellular electrograms were recorded with a 208-point multi-electrode array, mounted on a micromanipulator. Electrodes were silver wires of 0.1mm diameter and arranged in a 16x13 grid at 0.5mm inter-electrode distances. Recordings were made in unipolar mode with regard to a reference electrode connected to the support of the heart. Electrograms were acquired with a 256-channel data acquisition system. Signals
were filtered (DC-1kHz [6dB]) and digitized with 24-bit resolution and 2kHz sampling frequency. The input noise of the system was 4µV (peak-peak). Recordings were made during stimulation (rectangular pulses of 2ms duration and twice diastolic threshold) from the center of the grid at a basic cycle length of 200ms. The ERP, i.e., the coupling interval of the longest premature stimulus that failed to activate the entire heart, was determined by applying 8 stimuli at a basic cycle length of 200ms followed by 1 premature stimulus. Starting at 180ms, the coupling interval of the premature stimulus was first reduced in 10ms steps until conduction block occurred; subsequently, starting from the coupling interval of the last conducted premature stimulus, the coupling interval was prolonged with 1ms steps until ERP was reached. The time of steepest negative dV/dt in the unipolar electrogram was used as activation time and determined for all signals. Activation maps were constructed from the activation times. Longitudinal and transversal conduction velocities and activation wavelengths were calculated as described previously.

The susceptibility to arrhythmias was tested as follows. Eight basic stimuli were applied followed by 1 premature stimulus at a coupling interval 5ms longer than the locally determined ERP. If this protocol failed to induce arrhythmias, we applied 8 basic stimuli followed by 4 premature stimuli at ERP+5ms. If no arrhythmias could be induced, 10-second burst pacing at the shortest possible cycle length was applied. We first determined ERP and susceptibility to arrhythmias in RV and subsequently in LV.

**Myocyte isolation and measurement of cell dimensions**

Enzymatically isolated myocytes were placed in a cell chamber on the stage of an inverted microscope and superfused with a solution (37°C) containing (mM): NaCl 148, NaHCO₃ 1.0, KHCO₃ 3.3, KH₂PO₄ 1.4, MgCl₂ 1.9, CaCl₂ 1.3, glucose 11.0, HEPES 16.8, pH=7.3 (NaOH). The length and width of 50 randomly selected viable rod-shaped myocytes of RV and LV each of 7 RVF rats and 4 controls were measured (3µm resolution).
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Cellular electrophysiology

Data acquisition. APs and whole-cell sodium current ($I_{Na}$) were recorded using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Voltage control, data acquisition, and analysis were accomplished using custom-made software. $I_{Na}$ signals were low-pass filtered (cut-off 5kHz) and digitized at 20kHz. APs were filtered and digitized at 10 and 40kHz, respectively. Cell membrane capacitance ($C_m$) was estimated as described previously 9. Series resistance was compensated for by ≥80%.

Action potential. APs were recorded at 36±0.2°C with the perforated patch-clamp technique. The bath solution contained (mM): NaCl 140, KCl 5.4, CaCl$_2$ 1.8, MgCl$_2$ 1, glucose 5.5, HEPES 5; pH 7.4 (NaOH). Pipettes (2–3 MΩ) were filled with solution containing (mM): K-gluconate 125, KCl 20, NaCl 5, amphotericin-B 0.22, HEPES 10; pH 7.2 (NaOH). APs were elicited during stimulation at a frequency of 1.0, 2.5, 5.0, and 7.5Hz by 3-ms 20-40% suprathreshold current pulses through the patch pipette. We analyzed resting membrane potential, maximal AP upstroke velocity, maximal AP amplitude, and AP duration at 20, 50, and 90% repolarization (APD$_{20}$, APD$_{50}$, and APD$_{90}$). Data from 10 consecutive APs were averaged. Potentials were corrected off-line for their estimated liquid junction potential.

Sodium current. $I_{Na}$ was recorded at 20°C with the whole-cell ruptured patch-clamp technique. The bath solution contained (mM): NaCl 7.0, CsCl 133, CaCl$_2$ 1.8, MgCl$_2$ 1.2, glucose 11.0, HEPES 5.0, and nifedipine 0.005; pH 7.4 (CsOH). Pipettes (1–2 MΩ) were filled with solution containing (mM): NaCl 3.0, CsCl 133, MgCl$_2$ 2.0, Na$_2$ATP 2.0, TEACl 2.0, EGTA 10, HEPES 5.0; pH 7.3 (CsOH). Activation and inactivation were determined using the protocol indicated in Figure 4A, with a holding potential of -140 mV and a 3sec cycle time. Current density was calculated by dividing whole-cell current amplitude by $C_m$. Voltage-dependence of (in)activation was determined by fitting a Boltzmann function ($y=A/[1+\exp((V-V_{1/2})/k)]$) to the individual curves, yielding half-maximal voltage ($V_{1/2}$) and slope factor (k).
Histology
RV and LV tissues of 4 RVF rats and 4 controls were used for histologic investigation. Tissue was excised from the positions where the multielectrode recordings were made and fixed in formalin. Seven-µm thick sections were cut parallel to the epicardium, stained with Picro-Sirius Red, and examined by light microscopy (10x magnification) for collagen. The amount of collagen in the recording area was determined using the Image-Pro Plus software package (version 5.02, Media Cybernetics) after digitizing 6 randomly selected fields per section with a slide scanner.

Western blots and immunohistochemistry
Total cellular protein was isolated from the RV and LV free walls of 5 RVF rats and 5 controls. Tissue sections were rapidly frozen in liquid nitrogen, pulverized in a custom-made mortar cooled with liquid nitrogen, and transferred to lysis buffer (RIPA buffer with 150mM NaCl, 10mM Na₂HPO₄, 1% Triton X-100, 1% deoxycholic acid, 0.1% SDS, 1 mM EDTA, 50mM NaF, 0.5% 1.5mM aprotinin, and 0.5% 200mM PMSF in isopropanol). Total protein content of the supernatant was analyzed with a BCA quantification assay. Equal amounts of the individual lysates were pooled for each group (n=5). SDS-PAGE and Western blotting were performed using antibodies directed against the Connexin-43 protein (Cx-43) (Transduction).

To compare the expression levels of Cx-43 between RVF and control hearts, the pooled samples were separated on gel (25µg total cellular protein per sample) and blotted onto nitrocellulose. The optical densities of the obtained signals were quantified (ImageQuant software) and corrected for possible deviations in the amount of transferred total protein. For this, reversible Ponceau-S staining of the blots was performed after which signals were digitized by scanning at 400DPI.

Cryosections (10µm thickness) were sliced perpendicular to the epicardial surface from biopsies rapidly frozen in liquid nitrogen. Thus, each section encompassed
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the entire ventricular wall. Immunohistochemistry was performed as described previously\(^7\). In brief, sections were mounted on aminopropyltriethoxysilane-coated glass slides. For immunolabeling, sections were permeabilized in 0.2% Triton X-100 (1hr), blocked in 2% bovine serum albumin (30min) and incubated overnight with Cx43 (Zymed, 1:250) and α-actinin (Sigma, 1:1000) in the presence of 10% normal goat serum. Secondary labelling (2hr in presence of 10% normal goat serum) was performed with donkey-anti-mouse Texas Red (Jackson Labs, 1:100) and goat-anti-rabbit fluorescein isothiocyanate (Jackson Labs, 1:250). All chemicals were dissolved in phosphate buffered saline that also was used to wash the sections in between the subsequent incubations. Finally, sections were mounted in Vectashield mounting medium (Vector Laboratories) and examined with a light microscope with epifluorescence equipment.

Statistics
Statistical analysis was performed using SPSS software version 16.0.0. All data are presented as mean±SEM. Between-group comparisons were performed using unpaired two-tailed Student’s t tests. \(p\leq0.05\) was considered statistically significant.
Chapter 6

**Results**

Assessment of arrhythmias in CTEPH patients

The clinical, electrocardiographic and hemodynamic characteristics of the patients are presented in Table 1. The average duration of CTEPH symptoms was 3.2 years (range 0.6-12 years). At the time of referral to our hospital, most patients had severe CTEPH with marked reduction in exercise capacity, and RV contractile failure. All patients were in sinus rhythm, except for 1 patient (1%) who had permanent atrial fibrillation. Three patients (2%) had syncope; in 2 patients this was ascribed to paroxysmal atrial fibrillation. Ten patients (8%) complained about palpitations during daily physical activity, but ECG documentation of these episodes was not available. Among the 55 patients who underwent cardiopulmonary exercise testing, solitary supraventricular and ventricular extrasystoles were documented during exercise in 11 (20%) and 13 (24%), respectively. These ventricular extrasystoles had a left bundle branch block morphology/inferior axis (8 patients, 15%) or a right bundle branch block morphology/superior axis (5 patients, 9%). No VT/VF episodes or cardiac arrest were documented, neither at rest, nor during exercise testing.

Echocardiographic features and pathological findings in rat model

On average, RVF occurred at 26±2 (22-30) days after MCT injection. Table 2 shows echocardiographic parameters (recorded during the last examination before electrophysiologic study) and morphometric parameters of control and RVF rats. Compared to controls, RVF rats had significantly thicker RV wall, dilated RV with reduced contractility (reduced tricuspid annulus plane systolic excursion), and RV diastolic dysfunction. Diastolic function of LV was also impaired (reduced mitral E/A ratio). Echocardiographic data were in line with pathological findings: compared to controls, RVF rats had significantly reduced body, LV, and liver weights, and increased lung and RV weights. Moreover, RVF rats had pleural effusion, while controls did not.
Table 2: Echocardiographic and morphometric parameters of control and RVF rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RVF</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n=14</td>
<td>n=13</td>
<td></td>
</tr>
<tr>
<td><strong>Echocardiographic parameters at sacrifice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Right ventricle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV free-wall thickness, mm</td>
<td>0.63±0.01</td>
<td>1.12±0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV end-diastolic diameter, mm</td>
<td>4.1±0.1</td>
<td>6.9±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tricuspid E/A ratio</td>
<td>0.7±0.1</td>
<td>0.2±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tricuspid annulus plane systolic excursion, mm</td>
<td>3.1±0.3</td>
<td>1.6±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Left ventricle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior wall thickness, mm</td>
<td>1.54±0.07</td>
<td>1.71±0.09</td>
<td>0.14</td>
</tr>
<tr>
<td>End-diastolic diameter, mm</td>
<td>7.5±0.08</td>
<td>4.6±0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitral E/A ratio</td>
<td>1.25±0.02</td>
<td>0.84±0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>69.4±1.8</td>
<td>69.7±2.9</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>Morphometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>352±17</td>
<td>282±13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV weight, g</td>
<td>0.23±0.00</td>
<td>0.41±0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV, g</td>
<td>1.13±0.27</td>
<td>0.95±0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV weight/LV weight ratio</td>
<td>0.2±0.0</td>
<td>0.4±0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.3±0.1</td>
<td>2.5±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>15±1</td>
<td>10±1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

LV: left ventricle; RV: right ventricle; RVF: right ventricular failure.

Electrophysiologic characteristics of RVF and control rats

Ventricular arrhythmia inducibility

VT/VF was non-inducible during electrophysiologic study, neither in RVF rats (n=13), nor in controls (n=14).
Figure 1: Examples of determination of effective refractory period (A) during programmed stimulation and subepicardial activation maps during constant pacing at basic cycle length of 200 ms (B). RV: right ventricle; LV: left ventricle; S₁: stimulus applied at basic cycle length of 200 ms; S₂: premature stimulus with a coupling interval at the refractory period; RVF: RV failure; CTRL: control. (For color figure: see Appendix, page 253).
Right ventricular failure secondary to pressure overload is not associated with ventricular arrhythmias because wavelength is prolonged.

**Effective refractory periods and conduction velocities**

RVF rats had significantly longer RV ERP than controls (Figures 1A, 2A). LV ERP in RVF rats was also significantly longer than in controls, but the prolongation was less prominent than in RV (Figures 1A, 2A). RV longitudinal and transversal conduction velocities in RVF rats were significantly higher than in controls (Figures 1B, 2B-C), while the anisotropy ratio (ratio between longitudinal and transversal conduction velocities) was reduced (1.61±0.05 in RVF [n=13] vs. 1.90±0.06 in controls [n=14], p=0.002). In the LV of RVF rats, only longitudinal conduction velocity was significantly reduced (Figures 1B, 2C), and the anisotropy ratio was not significantly changed. Excitation wavelengths in both RV and LV were longer in RVF rats than in controls (Figure 2D).

**Action potential characteristics**

Figure 3A shows representative APs measured at a 2.5Hz pacing rate from a RV and LV myocyte of a control and RVF rat. Figure 3B shows average values of various AP characteristics. RV and LV myocytes of RVF rats had 300% and 50% longer APs at 90% repolarization, respectively. This AP prolongation was present at all pacing rates used (Figure 3C), but less pronounced at higher frequencies. In contrast, no significant differences in resting membrane potential, AP amplitude and maximal upstroke velocity were observed between RVF rats and controls (Figure 3B).

**Sodium current properties**

Figure 4A shows representative $I_{Na}$ recordings from a RV and LV myocyte of a control and RVF rat. Mean $I_{Na}$ densities were not significantly different between RVF rats and controls (Table 3). Voltage dependence of $I_{Na}$ activation was not different between RVF rats and controls in LV myocytes (Table 3, Figure 4B). However, in RV myocytes, $V_{1/2}$ of activation was ~9mV more negative in RVF than in controls ($p<0.05$, Table 3, Figure 4B). Voltage-dependent inactivation was not different between RVF rats and controls (Figure 4B).
Table 3: Parameters of sodium current.

<table>
<thead>
<tr>
<th></th>
<th>RV</th>
<th>LV</th>
<th>RVF</th>
<th>LVF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RV</td>
<td>Control</td>
<td>RVF</td>
</tr>
<tr>
<td>Current density, pA/pF</td>
<td>-86±11</td>
<td>-66±8</td>
<td>-76±11</td>
<td>-66±10</td>
</tr>
<tr>
<td>Inactivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{1/2}$, mV</td>
<td>-88.1±3.2</td>
<td>-95.5±2.7</td>
<td>-92.8±3.0</td>
<td>-89.6±4.0</td>
</tr>
<tr>
<td>$k$, mV</td>
<td>-6.7±0.5</td>
<td>-6.9±0.5</td>
<td>-6.6±0.6</td>
<td>-7.0±0.4</td>
</tr>
<tr>
<td>Activation</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$V_{1/2}$, mV</td>
<td>-52.0±2.5</td>
<td>-60.8±1.8*</td>
<td>-58.0±3.0</td>
<td>-58.5±2.2</td>
</tr>
<tr>
<td>$k$, mV</td>
<td>4.0±0.3</td>
<td>4.0±0.5</td>
<td>4.6±0.5</td>
<td>4.0±0.5</td>
</tr>
</tbody>
</table>

LV: left ventricle; RV: right ventricle; RVF: right ventricular failure; $V_{1/2}$: half-maximal voltage of (in)activation; $k$: slope factor of (in)activation. *p<0.05 vs. control.

Figure 2: Effective refractory period (A), longitudinal (B) and transversal (C) conduction velocities, and excitation wavelengths (D). RV: right ventricle; LV: left ventricle; CV: conduction velocity; ERP: effective refractory period; RVF: RV failure; CTRL: control.
Right ventricular failure secondary to pressure overload is not associated with ventricular arrhythmias because wavelength is prolonged.

**Figure 3:** Typical action potentials (APs) at 2.5Hz (A), average AP characteristics 2.5Hz (B), and average AP duration at 90% repolarization (APD90) at 1-8 Hz (C). dV/dt_{max}: maximal AP upstroke velocity; RMP: resting membrane potential; APA: AP amplitude. APD_{20} and APD_{50}: AP duration at 20 and 50% repolarization; RV: right ventricle; LV: left ventricle; RVF: RV failure; CTRL: control.
**Figure 4:** Typical examples of sodium current recordings (A) and average activation/inactivation curves (B). Inset shows the voltage clamp protocol used. RV: right ventricle; LV: left ventricle; RVF: RV failure; CTRL: control.
Tissue characteristics of RVF and CTRL rats

Cell dimensions
RV myocytes were wider in RVF rats than in controls (27.7±0.5 vs. 23.1±0.3µm, p=0.015), while their lengths were not significantly different (108.4±0.9 vs. 110.9±0.5µm, p=0.9). RV myocyte membrane capacitance was larger in RVF rats (26 myocytes, 166±9pF) compared to controls (46 myocytes, 132±6pF, p=0.002). In contrast, LV myocytes were shorter and narrower in RVF rats than in controls (111.6±0.7 vs. 122.0±0.4µm, p=0.02, and 21.9±0.2 vs. 25.3±0.3µm, p=0.002, respectively), but their membrane capacitances were similar (30 RVF myocytes, 144±6pF vs. 35 control myocytes, 143±7pF, p=0.9).

Expression and distribution of Connexin-43 protein
Figure 5A shows double labeling of Cx-43 and α-actinin. Cx-43 expression was homogeneous and aligned along the intercalated disc, and, to a lesser extent, along the lateral myocyte borders. Cx-43 expression in RVF rats was denser in RV and less dense in LV compared to controls, but remained homogeneous. Quantification of Cx-43 protein expression by Western blotting indicated a 30% increase in RV and a 24% decrease in LV of RVF rats (signals were corrected for differences in the total amount of transferred protein, Figure 5B).

Collagen content
The amount of interstitial collagen deposition was increased in RV, but not LV, of RVF rats compared to controls (Figure 5C).
Discussion

We found that the incidence of VT/VF in patients with CTEPH was low. In accordance with this finding, VT/VF was not inducible in our experimental RVF model. Detailed electrophysiologic analysis in this experimental model revealed that this protection from VT/VF was consistent with an increase in excitation wavelength of RV and LV. In RV, this increase was caused by faster conduction velocity and longer ERP. In LV, it mainly resulted from ERP prolongation.

*Incidence of ventricular tachyarrhythmias in pressure overload-induced RV failure*

Although atrial tachyarrhythmias are the most common arrhythmias encountered in patients with RVF, VT/VF may also occur in patients with RVF\(^2\). For example, patients with congenital heart defects who underwent corrective cardiac surgery are at increased risk for VT/VF\(^2,6\). However, these tachyarrhythmias are often linked to the surgical scars\(^21\), rather than to the presence of RV overload/failure. Accordingly, previous clinical\(^5\) and experimental\(^6\) studies have shown that RV pressure overload did not result in increased incidence or susceptibility to VT/VF. Similarly, in the present study, no episodes of clinically relevant ventricular tachyarrhythmias were documented in any CTEPH patient. Yet, CTEPH manifested in 3 patients as syncope, a common sign of pulmonary embolism\(^22\). While syncope was ascribed to atrial fibrillation in 2 patients, ventricular tachyarrhythmias cannot be excluded as a possible cause. Solitary ventricular extrasystoles with left bundle branch block and right bundle branch block morphologies were observed in 13 (24%) patients at \~2.5 and \~6.0 min after starting cardiopulmonary exercise testing. On the other hand, the reported arrhythmias, as present in 14% of syncope in pulmonary embolism, were associated with atrioventricular conduction disorders\(^22\).
Right ventricular failure secondary to pressure overload is not associated with ventricular arrhythmias because wavelength is prolonged

**Determinants of RV excitation wavelength**

Previous clinical\(^{11}\) and experimental\(^{10, 23, 24}\) studies have indicated that RV disease is associated with prolongation of RV AP duration. We also found AP prolongation and ~2-fold ERP increase in RV of RVF rats. These changes in repolarization are most likely due to cardiac ion channel remodeling, e.g., downregulation of inwardly rectifying potassium current (I\(_{\text{K1}}\)), transient outward potassium current (I\(_{\text{to}}\)), and/or the slow component of the delayed rectifier potassium current (I\(_{\text{Kr}}\))\(^{11}\). Conduction velocity, another determinant of excitation wavelength\(^{18}\), was faster in RV of RVF rats than in controls. RV myocyte hypertrophy (~22% larger cell width and ~26% larger cell capacitance in RVF rats) may explain this increase in conduction velocity\(^{16, 24-26}\). Although we also found a ~2-fold increase in interstitial collagen deposition that could theoretically impair transverse conduction, such a mild increase has been shown to hardly impair impulse propagation\(^{18, 27}\). Moreover, the observed ~30% increase in Cx-43 protein expression, another important determinant of impulse propagation\(^{27}\), would facilitate conduction. Finally, the parallel shifts in voltage dependent activation and inactivation are expected to counterbalance each other with regards to their effects on conduction velocity. In agreement with this expectation, maximal AP upstroke velocity was unchanged. In disagreement with our findings, Uzzaman et al. demonstrated that rats with MCT-induced RV hypertrophy had reduced longitudinal conduction velocity and unchanged transversal conduction velocity and Cx-43 protein expression\(^{28}\). These discrepancies may be due to the following. Firstly, Uzzaman et al. studied extracellular electrograms from an arterially perfused isolated RV free wall preparation, while we studied Langendorff-perfused hearts. Secondly, Uzzaman et al. studied rats at fixed time points (2 and 4 weeks) after MCT injection, although the rate of development of RVF in this model is highly variable\(^{15}\). Thus, these rats may have been at different stages of cardiac remodeling. Previous studies have shown both an up-regulation and down-regulation of Cx-43 protein expression during development of heart failure in man\(^{29}\). This may be due to differences in disease stages studied, and the fact that these changes in Cx-43 protein expression can be faithfully monitored thanks to the rapid turnover (half-life ~1.3 hours)
of Cx-43 protein. Consistent with this notion, Tritthart et al. observed slower conduction velocity when RV myocytes were only slightly hypertrophied, but faster conduction when hypertrophy was more pronounced. The primary goal of our investigation was to study electrophysiologic remodeling only at the end stage of RV disease, i.e., RVF.

**Determinants of LV excitation wavelength**

We found electrophysiologic remodeling in LV of RVF rats, i.e., AP/ERP prolongation, and longitudinal conduction slowing. Despite a reduced conduction velocity, the excitation wavelength in LV increased, similar to RV. In patients with CTEPH and MCT-injected rats with RV hypertrophy and failure, LV diastolic function is impaired due to underfilling. Indeed, the only echocardiographic LV variables that we found to be abnormal in RVF rats reflected diastolic dysfunction (LV end-diastolic diameter and E/A ratio), suggesting altered filling, while systolic parameters were unchanged. Mechanical underloading reduces cell dimensions, regardless of enhanced neurohumoral stimulation. Accordingly, length and width of myocytes from the LV lateral wall of RVF rats were ~10% smaller than in controls, and LV weight was reduced, similar to previous studies. Reduced cell dimensions may explain the ~10% reduction in longitudinal conduction velocity. Further evidence of LV changes in RVF is provided by a pattern of fetal gene expression in LV similar to that of RV in two different rat models of RV disease: MCT-treated and pulmonary artery banded rats. Since it is known that the unloaded heart replicates fetal gene expression profiles of hypertrophy and exhibits a hypertrophic phenotype of repolarization, we speculate that LV electrophysiologic remodeling in RVF due to pressure overload may be secondary to chronic underfilling. The cellular mechanisms responsible for such remodeling in mechanically underloaded LV in RVF are unexplored.
Right ventricular failure secondary to pressure overload is not associated with ventricular arrhythmias because wavelength is prolonged.

Figure 6: Expression and distribution of Connexin-43 and interstitial collagen deposition. **A:** Immunolabeling of Connexin-43 (green) (upper panel) and double labeling of Connexin-43 (green) and α-actinin (red) (lower panel); **B:** Western blot (upper panel) and Connexin-43 expression (lower panel); **C:** Picro-sirius Red staining of collagen (upper panel) and interstitial collagen content (lower panel). Scale bars in panels A and C: 100µm and 50µm, respectively. RV: right ventricle; LV: left ventricle; RVF: RV failure; CTRL: control. Cx-43: Connexin-43. (For color figure: see Appendix, page 254).

Arrhythmia mechanisms in RV failure

Besides re-entry, early afterdepolarizations and delayed afterdepolarizations may underlie ventricular tachyarrhythmias in heart failure. AP prolongation, found in the present study, and abnormal cellular calcium handling, reported previously in this model, generally favor early afterdepolarizations and delayed afterdepolarizations, respectively.
Early afterdepolarizations are not likely to occur because they require slow heart rates\textsuperscript{13}, whereas heart rates were fast in our RVF rats\textsuperscript{15}. The finding that burst pacing failed to induce ventricular tachyarrhythmias indicates low susceptibility to delayed afterdepolarizations. While we found low susceptibility to ventricular tachyarrhythmias in RVF, clinical studies have indicated that supraventricular tachyarrhythmias, particularly atrial fibrillation, are common\textsuperscript{44}. Indeed, in the present study, 8% of our patients had supraventricular tachyarrhythmias at rest, while 20% had solitary supraventricular extrasystoles during exercise testing. Given that atrial fibrillation is highly dependent on sufficient shortening of the excitation wavelength\textsuperscript{9}, these findings suggest that excitation wavelength in atrium is shorter during RVF. We did, however, not conduct atrial electrophysiologic studies to test this possibility.

Although RV and LV failure from pressure overload share features of electrophysiologic remodeling (AP prolongation and increased conduction velocities caused by increased myocyte dimensions\textsuperscript{16, 24}), ventricular arrhythmias, while rare in RVF, occur frequently in LV failure\textsuperscript{1, 8}. A possible explanation for this RV-LV discrepancy is the fact that, in LV failure, Cx-43 protein is down-regulated and/or heterogeneously distributed\textsuperscript{45-47}. In contrast, we found that, in subepicardial layer of RV, Cx-43 protein expression was increased and had homogeneous tissue distribution.

Study limitations
The retrospective design of our clinical study may have led to underestimation of the incidence of ventricular tachyarrhythmias in CTEPH. Possible occurrence of tachyarrhythmias was assessed periodically by clinical examination and standard ECGs. Because of the lack of closer rhythm monitoring in CTEPH (e.g., periodical Holter monitoring) and ECG monitoring in animals, it is possible that self-limiting VT episodes may have been missed. However, no episodes of clinically relevant tachyarrhythmias in patients were probably missed. Similarly, Sanyal et al. did not find VT episodes in MCT rats with RVF using telemetry ECG monitoring\textsuperscript{48}. Moreover, exercise testing did not evoke ventricular tachyarrhythmias in our CTEPH patients, although this stressor often
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causes significant arrhythmias in patients with structural heart disease. Another possible limitation is that detailed electrophysiologic analysis, as presented here, required the use of experimental animals. Although findings from the MCT rat model may not necessarily be applicable to CTEPH patients, this model is a widely accepted model to obtain pathophysiologic insights into pressure overload-induced RVF. This is relevant, as 97 patients (80%) in the present study were in NYHA functional class III/IV.

**Conclusion**
The incidence of ventricular tachyarrhythmias in patients with severe CTEPH is low. In parallel, susceptibility to ventricular tachyarrhythmias in the MCT rat model of pulmonary hypertension-induced RV disease is low. This is associated with increased excitation wavelength in both ventricles, which protects the heart against the occurrence of re-entrant excitation.

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**Disclosures**
None
References


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