Right ventricular pacing improves right heart function in experimental pulmonary arterial hypertension: a study in the isolated heart


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Running head: RV-pacing in experimental pulmonary hypertension

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ABSTRACT

Right heart failure in pulmonary arterial hypertension (PH) is associated with mechanical ventricular dyssynchrony, which leads to impaired right ventricular (RV) function, and - by adverse diastolic interaction - to impaired left ventricular (LV) function as well. However, therapies aiming to restore synchrony by pacing are currently not available. In this proof-of-principle study, we determined the acute effects of RV-pacing on ventricular dyssynchrony in PH.

Chronic PH with right heart failure was induced in rats by injection of monocrotaline (80 mg/kg). To validate for PH-related ventricular dyssynchrony, rats (6 PH, 6 controls) were examined by cardiac magnetic resonance imaging (9.4 Tesla), twenty-three days after monocrotaline- or sham-injection. In a second group (10 PH, 4 controls), the effects of RV-pacing were studied in detail, using Langendorff-perfused heart preparations.

In PH, septum bulging was observed, coinciding with a reversal of the trans-septal pressure gradient, as observed in clinical PH. RV-pacing improved RV systolic function, compared to unpaced condition (RV \(dP/dt_{max}\): +8.5±1.3 %, \(p<0.001\)). In addition, RV-pacing markedly decreased PTI_{RV-P-LVP}, an index of adverse diastolic interaction (-24±9 %, \(p<0.01\)), and RV-pacing was able to resynchronize time of RV and LV peak-pressure (\(\Delta t_{peak}\), unpaced: 9.8±1.2 ms vs. paced: 1.7±2.0 ms, \(p<0.001\)). Finally, RV-pacing had no detrimental effects on LV function or coronary perfusion, and no LV pre-excitation occurred.

Taken together, we demonstrate that in experimental PH, RV-pacing improves RV function and diminishes adverse diastolic interaction. These findings provide a strong rationale for further in vivo explorations.
Keywords

Pulmonary hypertension; right ventricular dysfunction; artificial cardiac pacing; magnetic resonance imaging; Langendorff preparation
**INTRODUCTION**

Pulmonary arterial hypertension (PH) is characterized by progressive pulmonary vascular remodeling. During the progression of the disease, right ventricular (RV) afterload continues to rise and eventually right heart failure develops in the majority of patients.

In PH-patients, signs of mechanical RV *dyssynchrony* along with signs of adverse interventricular *diastolic interaction* are often observed. This results in inefficient pumping of the heart. In essence, the duration of RV contraction is lengthened due to increased RV afterload. As a consequence, time-to-peak shortening of the RV free wall is delayed, even beyond closure of the pulmonary valves. Loss of a coordinated ventricular contraction results in impaired RV systolic function. In addition, the prolonged RV contraction in early left ventricular (LV) diastole causes the already relaxing interventricular septum to bulge into the left ventricle. This negatively influences early LV filling, eventually contributing to the impairment of LV diastolic function as well.

To this date, no specific treatment is available for the failing right ventricle. Cardiac resynchronization therapy is a well-established treatment for LV dyssynchrony related to left heart failure, and might be an interesting therapeutic option for right heart failure as well. However, we recently demonstrated that the origin of PH-related ventricular dyssynchrony lies in regional differences in the duration of the contraction, rather than regional differences in onset of the contraction (e.g. due to a conductance delay). For this reason, PH-related ventricular dyssynchrony is essentially different from dyssynchrony associated with left heart failure.

In the present *proof-of-principle* study, we tested whether RV-pacing could synchronize pressure generation across the septum, resulting in an improvement of RV systolic function and a reduction of adverse diastolic interaction. First, we validated the monocrotaline rat model, a well-established model for chronic PH, for the presence of ventricular dyssynchrony.
Subsequently, we evaluated the acute effects of RV-pacing on cardiac performance and PH-related ventricular dyssynchrony in isolated Langendorff-perfused heart preparations. This approach allows relatively easy manipulation and offers a high degree of preparation stability in which LV and RV load can be varied independently, with derivation of cardiac-specific functional data. (20)
MATERIALS AND METHODS

All experiments were approved by the Institutional Animal Care and Use Committee of the VU University Amsterdam.

Experimental model of pulmonary arterial hypertension

In total, 26 male Wistar rats were included in the study (150-175g; Harlan, Horst, the Netherlands). PH was induced (n=16) by a single subcutaneous injection of monocrotaline (MCT; 80 mg/kg dissolved in sterile saline; Sigma-Aldrich, Zwijndrecht, The Netherlands). This resulted in a PH-phenotype, followed by right heart failure ~23 days after injection.(8; 15) The control group was injected with saline only (n=10).

Cardiac magnetic resonance imaging

Twenty-three days after MCT-injection, the presence of ventricular dyssynchrony in vivo was assessed in PH-rats and compared to controls (6 for each group), by measuring cardiac function and the behavior of the interventricular septum with cardiac magnetic resonance imaging (CMR; 9.4 Tesla; Varian Medical Systems, Palo Alto CA), as previously described.(26) After CMR-scanning, all rats were euthanized and their organs weighed. Images were analyzed off-line using Segment (version 1.698; http://segment.heiberg.se).(16) Endocardial borders of both ventricles were automatically detected for all slices of the heart and for each frame in the heart cycle. LV and RV volume curves were constructed using the modified Simpson’s rule, from which end-diastolic and end-systolic volume, peak filling rate, stroke volume, heart rate, cardiac output, and ejection fraction were derived.(11; 12) Septum curvature was calculated as previously described.(25) In short, the anterior, middle and posterior positions of the interventricular septum at midventricular level were determined,
through which a circle was fitted. The reciprocal of the radius of this circle was used to quantify septum curvature (1/R), and was defined positive if the septum bowed toward the right ventricle.

**Isolated Langendorff-perfused heart preparation**

To characterize the hearts of PH-rats and controls (10 PH-rats, 4 controls; no CMR performed), and to study ventricular dyssynchrony in detail, a Langendorff-setup was used as previously described, with balloons in left and right ventricle (Fig.1).(20) The heart was perfused (at 35-37° C) using a modified Krebs-Henseleit solution (composition in mM: 118.5 NaCl, 4.7 KCl, 1.4 CaCl₂, 25 NaHCO₃, 1.2 MgCl₂, 1.2 KH₂PO₄, and 11 glucose) that was continuously gassed with 95% O₂ / 5% CO₂ (pH 7.4). Coronary perfusion pressure was set at a constant value of 80 mmHg to minimize edema formation.(27; 29) Electrodes were placed at the right atrium, and at a vessel-free area of the LV and RV free wall (LV: posterolateral - midventricular; RV: RV “anterolateral”, opposite to the LV electrode), after which normal atrial and subsequent ventricular activation were checked to verify that the intrinsic conductance system was intact. In addition, signals of LV and RV electrodes were compared to detect potential differences in electrical activation of left and right ventricle. Atrial and ventricular threshold stimuli were determined, and the heart was atrial-paced at 4.0 Hz (pulse duration 1.0 ms, at twice the threshold). During the whole experiment, electrical activity and stimuli, LV and RV pressures, and coronary flow were continuously recorded with a sample rate of 2.0 kHz.

After stabilization (10 min), the volumes at maximal developed pressure (Vₘₐₓ) of both ventricles were determined by small stepwise increases and decreases in balloon volume (in analogy to Lₘₐₓ, used in isolated muscle studies (31)), subsequently the pressure-volume (PV-)relationship of the left ventricle was determined (in the physiological range of 70 to 100%...
RV-pacing protocol

RV-pacing was only performed in PH-hearts; RV-pacing experiments in normal hearts were not performed, since it is known that this results in loss of synchrony and worsening of cardiac function.(30) LV volume was set at 75 %V\textsubscript{max} and RV volume was set at 95 %V\textsubscript{max}. These volumes correspond with LV and RV end-diastolic pressures of 5 and 10 mmHg respectively (Fig.2), as observed in PH-patients,(11) and in MCT-treated rats \textit{in vivo} (15) The intrinsic atrioventricular delay (AV-time) was defined as the time-interval between atrial and (right) ventricular activation (Fig.1).

RV-pacing was performed by direct stimulation of the RV free wall (using the RV electrode), triggered from atrial activation, starting with an AV-time equal to the intrinsic AV-time. Subsequently, AV-time was shortened in steps of 10 ms (AV-shortening). Effects of RV-pacing stabilized within 5 seconds. After the experiment, all hearts were dissected in left (including interventricular septum) and right ventricle, and weighed.

Functional assessment of RV-pacing

The isovolumic pressure recordings were evaluated off-line using MATLAB (version R2007b, The MathWorks, Natick MA). Signals were averaged over approximately hundred beats (25 seconds). The effects of RV-pacing on both intra- as well as inter-ventricular aspects of PH-related dyssynchrony were evaluated.(13) Intra-ventricular dyssynchrony was measured by RV dP/dt\textsubscript{max} and RV systolic pressure (RV SP). The time difference between RV and LV peak-pressure was used as an index for inter-ventricular dyssynchrony (Δt\textsubscript{peak}).
PTI_{RV>P>LVP} was used to quantify the interventricular diastolic interaction in isolated hearts. It measures the degree as well as the duration of reversed pressure differences across the interventricular septum (i.e. RV pressure > LV pressure) during a heart beat, which is considered the driving force that causes the septum to bulge into the left ventricle, (25) impairing LV filling. (12; 28) This parameter is especially sensitive (it decreases) for improvements in synchronic pressure generation across the septum (due to RV-pacing). PTI_{RV>P>LVP} expresses the pressure-time integral of the trans-septal pressure gradient when RV pressure exceeds LV pressure, and was calculated by:

\[ \text{PTI}_{RV>P>LVP} = \int_{1 \text{beat}} (RV \text{ pressure} - LV \text{ pressure}) \, dt, \text{ when RV pressure} - \text{LV pressure} > 0 \]

The onset of LV and RV contraction was defined as the time-point where pressure rose to 5% of developed pressure above diastolic pressure. The difference between the onset in RV and LV contraction was used to identify presence of LV pre-excitation (\(\Delta t_{\text{onset}}\)). LV pre-excitation refers to depolarization of the LV myocardium that is earlier than would occur by conduction of an impulse through the AV node (in this case LV depolarization triggered by artificial pacing of the RV free wall), and is known to be detrimental for LV function in the long-term. (30) This phenomenon can be recognized from pressure recordings, when the difference in onset no longer changes at larger AV-shortening intervals (see Fig.1, Fig.5D); in that case RV-pacing no longer solely advances RV contraction, but prematurely activates the left ventricle as well.

The duration of RV and LV contraction was defined as the time-interval between 5% rise and 95% fall in developed pressure. Coronary perfusion was measured by average total coronary flow.
Statistical analyses

All data were verified for normal distribution, and values were expressed as mean±SEM, unless stated otherwise. A p-value <0.05 was considered significant. Group differences were analyzed by unpaired Student T-test. Septum curvature, ventricular volume curves and PV-relationships were analyzed by two-way ANOVA for repeated measurements. Paired Student T-test was performed to evaluate the effect of RV-pacing.
RESULTS

**General characteristics of PH-rats vs. controls**

In PH-rats, CMR revealed significantly smaller cardiac output, stroke volume, lower heart rate and RV ejection fraction, and significantly larger RV end-diastolic volumes, compared to control (Table 1). Autopsy showed a significant increase in (wet) lung mass and RV / (LV+S) mass ratio (Table 1). An upward shift in systolic and diastolic PV-relationships for the right ventricle in PH was observed; The PV-relationships for the left ventricle were not different between PH and control (Fig.2). These results indicate PH-induced RV remodeling and RV dysfunction in MCT-treated rats.

**Septum bulging and PH-related ventricular dyssynchrony in vivo**

In PH-rats, CMR revealed that the interventricular septum at the midventricular level was less curved throughout the cardiac cycle, compared to controls (Fig.3A,B; average 1/R, PH: 0.50±0.15 cm⁻¹ vs. control: 2.07±0.05 cm⁻¹, p<0.001). In addition, solely in PH-hearts septum bulging was observed. At early LV diastole, the septum temporarily protruded into the left ventricle (negative 1/R; Fig.3A,B). Furthermore, we found significantly smaller LV end-diastolic volumes and lower LV peak filling rates for PH (Table 1).

**Reversed trans-septal pressure gradient and ventricular dyssynchrony in isolated hearts**

Pressure measurements revealed no differences in the onset between RV and LV contraction in PH-hearts (onset delay RV-to-LV, PH: +5.1±0.6 ms vs. control: +5.5±0.4 ms, n.s.). This finding was confirmed by the lack of a difference in electrical activation between right and left ventricle (activation delay RV-to-LV, PH: +0.1±0.8 ms vs. control: +0.7±1.0 ms, n.s.).
contrast, we found a prolonged duration of RV contraction in PH-hearts (Fig.3C; duration of RV contraction, PH: 197±3 ms vs. control: 168±4 ms, p<0.001). As a consequence, only in PH-hearts RV pressures were found to exceed LV pressures during late systole in the heart cycle, resulting in a temporary reversal of the trans-septal pressure gradient (Fig.3D).

When comparing in vivo septum measurements (Fig.3B) with the pressure measurements obtained in isolated hearts (Fig.3D), it was found that time of peak negative trans-septal pressure gradient (t = 66±2 %RR-interval) coincided with the occurrence of maximal septum bulging in PH (t = 64±2 %RR-interval).

In addition, we found that in PH-hearts, PTI_{RV>P>LVP} (an index of diastolic interaction) was RV-volume dependent, whereas this relationship was not observed in controls (Fig.4).

**Effects of RV-pacing**

The effects of RV-pacing in PH-remodeled hearts were studied at different AV-shortening intervals. The intrinsic AV-time at baseline (no pacing of the RV free wall) was 89±3 ms (AV-time, control: 92±3 ms). At maximal RV dP/dt_{max}, AV-shortening was found to be 15±2 ms (p<0.001), which was considered as the optimal pacing interval (Fig 5B). At this interval, there was no evidence for LV pre-excitation (Fig.5D), as LV pre-excitation only occurred at longer AV-shortening intervals (AV-shortening at the transition point to LV pre-excitation, = 32±2 ms; p<0.001 vs. optimal pacing interval).

Compared to baseline, pacing at optimal interval improved RV systolic function in all experiments; RV dP/dt_{max} (baseline PH: 1.75±0.04 *10^{3} mmHg/s, control: 0.88±0.08 *10^{3} mmHg/s) increased by 8.5±1.3 % (p<0.001; Fig.6A), and peak RV systolic pressure increased by 2.7±0.6 % (p<0.01; Fig.6B). Pacing also decreased the time-difference between RV and LV peak-pressure (Δt_{peak}, PH baseline: 9.8±1.2 ms vs. paced: 1.7±2.0 ms, p<0.001; Δt_{peak} control: -3.5±0.6 ms; Fig.6C). In addition, RV-pacing positively influenced the diastolic
interaction, as PTI_{RVP>LVP} decreased by 24±9 % (p<0.01; Fig.6D). Furthermore, RV-pacing shortened the duration of RV as well as LV contraction (RV duration at baseline: 197±2 ms, change in RV duration after pacing: -1.0±0.4 %, p<0.05; LV duration at baseline: 191±2 ms, after pacing: -1.3±0.3 %, p<0.001).

No significant effects of RV-pacing were observed on RV diastolic function (RV DP, baseline: 8.5±1.2 mmHg, paced +0.9±1.2 %; RV dP/dt\text{\textsubscript{min}}, baseline: -819±28 mmHg/s, paced: -2.0±2.2 %; both n.s.). Also, no effects of RV-pacing were observed, either on LV function (LV SP, baseline: 95±2.2 mmHg, paced: +0.2±0.3 %; LV DP, baseline = 2.4±0.4 mmHg, paced: -3.2±2.6 %; both n.s.), or on coronary perfusion (mean Qcor, baseline: 15±1 ml/min, paced: +0.3±0.3 %; n.s.).

The minimal rundown of the functional properties (decline in developed pressure: <5%/hr, increase in diastolic pressure: < 5%/hr), during our short-lasting experiment (<30 min), suggest minimal effect of edema formation.
DISCUSSION

After validation of the MCT-rat model for PH-related ventricular dyssynchrony, we demonstrated that in chronic PH:

1) RV-pacing improved RV systolic function, characterized by enhanced RV dP/dt$_{\text{max}}$ and peak RV systolic pressure.

2) RV-pacing diminished adverse interventricular diastolic interaction, by resynchronizing time between RV and LV peak-pressure ($\Delta$$_{\text{peak}}$), and reducing PTI$_{\text{RVP-LVP}}$.

In addition, RV-pacing slightly shortened the duration of RV as well as LV contraction; No (detrimental) effects on LV function or coronary perfusion were observed, and there was no evidence for LV pre-excitation.

By providing this proof-of-principle, the findings may give support for the potential role of RV-pacing as a novel treatment for PH-induced right heart failure.

Validation of the monocrotaline- rat model for ventricular dyssynchrony

The MCT-rat model is a well-established model for chronic PH in general,(34) but so far this model has not specifically been validated for PH-related ventricular dyssynchrony. Therefore, we first investigated whether signs of ventricular dyssynchrony were present in vivo.

We used sophisticated CMR-techniques to accurately quantify septum curvature and LV and RV function in the MCT-rat model. CMR offers superior imaging quality and allows generation of cross-sectional images in virtually any plane, both of major advantage, especially when visualizing septum bulging.(2) Our CMR measurements confirmed the pulmonary hypertensive state of the MCT-treated rats, with clear signs of right heart failure. Moreover, CMR demonstrated the presence of septum bulging, low LV end-diastolic volumes
and low LV peak filling rates, comparable to the clinical situation.(3; 22) As observed in clinical PH, ventricular dyssynchrony in MCT-induced PH was explained by regional differences in duration, rather than in onset of contraction (as measured by ECG and pressure recordings). Prolonged RV contractions and reversal of trans-septal pressure gradients in our PH-model were demonstrated by biventricular pressure measurements in isolated Langendorff-perfused heart preparations, which is in line with what was previously shown by Boissiere et al.(6) It is important to note that the expected coincidence in time of septum bulging and peak reversed trans-septal pressure gradient (RV pressure exceeds LV pressure; Fig.3) was also found in the isolated hearts.

These observations demonstrate that the MCT-model is an appropriate model to study PH-related ventricular dyssynchrony, and in addition, that ventricular dyssynchrony can be studied in detail in a Langendorff-setup with balloons in both ventricles. This approach has major advantages. It allows relatively easy manipulation with a high degree of preparation stability. Furthermore, it allows derivation of robust functional data with cardiac-specific parameters. Finally, in the Langendorff-setup LV and RV load can be varied independently, by changing ventricular volumes, which enabled us to reveal the relationship between ventricular dyssynchrony and RV load.

**Beneficial effects of RV-pacing**

To the best of our knowledge, our study is the first that explored the effects of RV-pacing on intra- and inter-ventricular dyssynchrony in chronic PH. We found a modest, but highly significant improvement in RV systolic function. Moreover, we observed an important reduction in interventricular diastolic interaction, without detrimental effects on LV function or coronary perfusion. At the optimal RV-pacing interval, there was no evidence for LV pre-excitation, which is known to be detrimental for LV function at the long-term.(30)
The early activation of the RV free wall probably compensated for the longer RV contraction period and therefore the delay in time-to-peak-shortening of the RV free wall relative to the interventricular septum and LV free wall.(6; 22) Pacing helped to restore synchrony of the left and right ventricle, and as a result RV systolic function improved. The time of activation is critical: if the RV free wall is activated too early synchrony is lost again, which explains the initial rise and then fall in RV $\frac{dP}{dt_{\text{max}}}$ with increasing AV-shortening (Fig.5B).

Another beneficial effect of pacing was the reduction in interventricular diastolic interaction, expressed by $\text{PTI}_{\text{RVP-LVP}}$. Pacing-induced earlier activation of the RV free wall and the shortened RV contraction resulted in an earlier start of RV relaxation. The partially restored synchrony in the relaxation of both ventricles explains the observed reduction of the $\text{PTI}_{\text{RVP-LVP}}$. Although isovolumic pressure measurements in our Langendorff-setup cannot directly provide this information, a marked decrease in $\text{PTI}_{\text{RVP-LVP}}$ together with a shortened duration of LV contraction would predict less septum bulging and improvement in early LV filling.(12; 28)

Recently, Quinn et al. also reported positive effects of pacing in a different model of RV pressure overload.(24) However, their findings are only partially applicable to the PH-patient group, because they applied an acute pressure overload in a pig-model with the conductance system artificially damaged by ethanol injection. A few clinical studies have explored the effect of RV-pacing on RV dysfunction secondary to congenital heart disease (studies on systemic right ventricles are not discussed here).(7; 10) These studies aimed to restore RV electromechanical dyssynchrony related to a complete right bundle branch block, a late complication of surgical repair. However as mentioned earlier, ventricular dyssynchrony in PH is based on prolonged contraction, rather than disturbances in the electrical conductance system.(22)
Limitations – the isolated heart vs. in vivo

This study supports the potential role of RV-pacing for the treatment of PH-related ventricular dyssynchrony and right heart failure. However, the results cannot be translated directly to the in vivo situation yet; future studies in a large animal model or (acute) RV-pacing experiments in PH-patients are necessary.

In the Langendorff preparation the mediatory role of RV afterload remains unknown, which limits the prediction of the effects of RV-pacing and improved RV contractility on stroke volume and cardiac output. Nonetheless, related studies reported an improvement of cardiac output after RV-pacing to the same extent as the improvement in RV contractility. (10; 24) RV-pacing could also potentially worsen tricuspid regurgitation through elevation of RV systolic pressures. On the other hand, it was recently shown that resynchronization therapy in left heart failure actually reduced pre-existing mitral regurgitation. (35)

Another important issue is the role of the pericardium. In our isolated Langendorff-perfused hearts, the pericardium was removed, which is known to reduce the interventricular diastolic interaction in the case of RV pressure overload. (4) This might explain why we did not observe a significant reduction in LV diastolic pressures / LV filling pressures by RV-pacing (that were already low at baseline) in our isolated heart preparations. (5) The effects of RV-pacing will probably be more pronounced in vivo with the pericardium intact.

Crystalloid based Langendorff-perfused hearts are prone to edema formation, which could affect diastolic properties. However, with a coronary perfusion pressure of 80 mmHg, this was reduced to a minimum. (27; 29) In addition, edema formation was found to have only limited functional effects during our experiments, as we observed a minimal rundown of the functional properties during our short-lasting experiments and, we were also able to detect clear differences in diastolic properties between control and PH-heart.
We found longer PR-intervals than are reported for (PH-)rats in vivo (~90 vs. ~60 ms).(17) However, no differences were observed between PR-intervals of isolated PH- and control hearts. We therefore conclude that the prolonged PR-interval, compared to the in vivo situation, is most likely to be attributed to the Langendorff set-up in general and unlikely to be related to differences in cardiac condition between PH-hearts and controls. Furthermore, the apparently prolonged PR-interval is of little relevance for the interpretation of our finding, as the intervention studied involves ventricular activation, which follows after the PR-interval. As a last point, clinical effective medical therapies, such as epoprostenol, are known to have a relatively small impact on hemodynamic measures, which nonetheless translate to improved survival.(1) Therefore, the small acute improvements in RV function found here, may in the long-term translate into substantial benefit.

Conclusions

In our experimental PH-model, RV-pacing improved cardiac performance through alleviation of PH-related ventricular dyssynchrony. The promising results of this study identify RV-pacing as a potential novel treatment for right heart failure in PH, and provide a strong rationale for future investigations evaluating the effects of RV-pacing in vivo.
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GRANTS

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DISCLOSURES

None.
REFERENCES


of either compensated ventricular hypertrophy or heart failure. *Physiol Genomics* 21:


FIGURES, FIGURE LEGENDS

Figure 1:
The Langendorff / RV-pacing setup. Ventricular balloons were inserted into both ventricles, volumes were set, and isovolumic LV and RV pressures measured (P_LV, P_RV). Epicardial electrodes were placed at the right atrium, LV and RV free wall. RV-pacing was performed by shortening atrioventricular delay (AV-shortening) with the use of a second (coupled) pulse generator. AV-time = time-interval between atrial (A) and ventricular (V) electrical activation.

Figure 2:
LV and RV pressure-volume relationships of PH-hearts and control hearts. A,C) Compared to control, a significant upward shift in PV-relationship of the right ventricle was found for both systole (RV SP) and diastole (RV DP) in PH-hearts, indicating PH-induced RV remodeling (LV volume set at 75% V_max). B,D) No differences in PV-relationship were observed for the left ventricle (RV volume set at 75% V_max). Data are presented as mean±SEM; PH: n = 10, control: n = 4. %V_max = volume percentage of the ventricular volume at maximal developed pressure.

Figure 3:
PH-related ventricular dyssynchrony in monocrotaline-treated rats. A) Examples of short-axis CMR-images at the midventricular level of control- and PH-hearts at time points t = 0, and t = 65 %RR-interval. Epi- and endocardial borders are indicated by the thin lines. The interventricular septum is highlighted by the dotted line. Crosses indicate anterior, middle and posterior positions of the interventricular septum that were used to calculate septum curvature (1/R). B) Septum curvature was less pronounced in PH vs. control throughout the heart cycle, and septum bulging (negative 1/R, arrow) was observed in all PH-hearts (mean±SEM, both groups: n = 6). C) Examples of LV and RV pressure recordings in isolated Langendorff–perfused hearts of control and PH, used to construct the trans-septal pressure gradient. D) In PH, RV contraction is prolonged and as a consequence RV pressure exceeds LV pressure at early LV diastole, causing a momentary reversal of the trans-septal pressure gradient (arrow). No reversal was observed in control hearts (mean±SEM, PH: n = 10, control: n = 4).
Figure 4:

Relationship between RV volume and interventricular diastolic interaction in PH. A) Examples of LV and RV pressure measurements in a PH-heart for different RV volume settings: RV70, RV95 = RV pressure curves at 70 or 95 %V_max (thick red lines; thin red lines represent RV pressure curves at intermediate RV volume settings). LV pressure was minimally affected, and for the sake of clarity only one LV pressure curve is shown. B) Only in isolated PH-hearts, interventricular dyssynchrony (quantified by PTI RVP>LVP; gray area) increased with increasing RV volume (mean±SEM, PH: n = 10, control: n = 4). PTI RVP>LVP = pressure-time integral, when RV pressure exceeds LV pressure.

Figure 5:

Determining the optimal RV-pacing setting. A) Example of a RV pressure curve at baseline (dashed line) and when optimally paced (solid line). For every experiment, the same series of AV-shortening were tested. Notice that pacing implies earlier start of RV contraction. B) RV dP/dt_{max} as function of AV-shortening. Optimal pacing interval was defined when RV dP/dt_{max} was highest. Notice the initial rise in RV dP/dt_{max} at short AV-shortening intervals and the subsequent fall at longer AV-shortening intervals. C) Diastolic interaction (PTI RVP>LVP) decreased precipitously with AV-shortening. Optimal RV dP/dt_{max} was found at relatively low values of AV-shortening. D) Difference in onset of RV vs. LV contraction (Δt_{onset}) as function of AV-shortening (overall, Δt_{onset} at LV-pre-excitation was -6.3±1.2 ms, ranging from -0.5 to -13.0 ms). LV pre-excitation only occurred at higher AV-shortening interval than when optimally paced.

Figure 6:

RV-pacing improved RV function and reduced interventricular diastolic interaction. A,B) At optimal pacing interval, RV-pacing significantly improved RV dP/dt_{max} and RV systolic pressure (RV SP), compared to baseline, and (partially) restored intra-ventricular synchrony. C) RV-pacing significantly reduced the time-difference between RV and LV peak (Δt_{peak}), and restored inter-ventricular synchrony. D) RV-pacing markedly decreased PTI RVP>LVP, an index of adverse diastolic interaction. Data are shown as mean±SEM (aside) and as paired individual observations (centre); n = 10 (all PH). Baseline, paced = before pacing, and when optimally RV-paced (AV-shortening = 15±2 ms).
### Table 1: General characteristics of PH-rats vs. controls

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### Autopsy

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<tr>
<th>PH</th>
<th>control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung mass (g)</td>
<td>2.14±0.12</td>
<td>1.06±0.09</td>
</tr>
<tr>
<td>Heart mass (g)</td>
<td>1.38±0.04</td>
<td>1.21±0.04</td>
</tr>
<tr>
<td>RV mass (g)</td>
<td>0.42±0.02</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>RV / (LV + S)</td>
<td>0.65±0.03</td>
<td>0.30±0.03</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>11.7±0.4</td>
<td>10.3±0.2</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>262±5</td>
<td>305±6</td>
</tr>
<tr>
<td>Tibia length (mm)</td>
<td>37.6±0.0</td>
<td>37.6±0.0</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM; n = 6 (both groups). RV / (LV + S) = RV over LV (including septum) mass ratio.
atrial pacing

SET

AV-time

right atrium

second pulse generator

RV free wall

monitoring electrogram

AV-time

AV-shortening

baseline

paced

time (ms)
CMR (in vivo)

A

<table>
<thead>
<tr>
<th>t = 0.0 * RR-interval</th>
<th>t = 0.65 * RR-interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>control</td>
</tr>
<tr>
<td>PH</td>
<td>septum bulging</td>
</tr>
</tbody>
</table>

B

1/R (cm⁻¹)

control

PH

p<0.001

time (%RR-interval)

Langendorff (isolated heart)

C

Pressure (mmHg)

<table>
<thead>
<tr>
<th>control</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
<td>RV</td>
</tr>
</tbody>
</table>

RV

Δ Pressure (mmHg)

<table>
<thead>
<tr>
<th>control</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
<td>RV</td>
</tr>
</tbody>
</table>

reversal trans-septal pressure gradient

p<0.001

time (%RR-interval)
AB
CMR (in vivo)

A

\[ t = 0.0 \times \text{RR-interval} \quad t = 0.65 \times \text{RR-interval} \]

control

PH

2.0 mm

septum bulging

LV

RV

PH

Langendorff (isolated heart)

C

Pressure (mmHg)

time (%RR-interval)

control

PH

RV

LV

D

\[ \Delta \text{Pressure (mmHg)} \]

time (%RR-interval)

control

PH

reversal trans-septal pressure gradient

p<0.001
A

RV pressure (mmHg) vs. time, relative to atrial activation (ms)

- Paced
- Baseline

B

RV \( \frac{dP}{dt} \max (10^3\text{mmHg/s}) \) vs. AV-shortening (ms)

- Optimally paced
- Baseline

C

PTV_{RV-LV} (mmHg*ms) vs. AV-shortening (ms)

- Baseline
- Optimally paced

D

\( \Delta t_{onset} \) (ms) vs. AV-shortening (ms)

- Baseline
- Optimally paced
- LV pre-excitation