Distinct loading conditions reveal various patterns of right ventricular adaptation

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Borgdorff MA, Bartelds B, Dickinson MG, Steendijk P, de Vroomen M, Berger RM. Distinct loading conditions reveal various patterns of right ventricular adaptation. Am J Physiol Heart Circ Physiol 305: H354–H364, 2013. First published May 31, 2013; doi:10.1152/ajpheart.00180.2013.—Right ventricular (RV) failure due to chronically abnormal loading is a main determinant of outcome in pulmonary hypertension (PH) and congenital heart disease. However, distinct types of RV loading have been associated with different outcomes. To determine whether the adaptive RV response depends on loading type, we compared hemodynamics, exercise, and hypertrophy in models of pressure overload due to pulmonary artery banding (PAB), pressure overload due to PH, combined pressure and volume overload, and isolated volume load. Ninety-four rats were subjected to either PAB, monocrotaline-induced PH (PH), aortocaval shunt (shunt), or combined monocrotaline and aortocaval shunt (PH + shunt). We performed pressure-volume analysis and voluntary exercise measurements at 4 wk. We compared PAB to PH (part I) and PH + shunt to either isolated PH or shunt (part II). In part I, enhanced contractility (end-systolic elastance and preload recruitable stroke work) was present in PH and PAB, but strongest in PAB. Frank-Starling mechanism was active in both PAB and PH. In PAB this was accompanied by diastolic dysfunction (increased end-diastolic elastance, relaxation constant), clinical signs of RV failure, and reduced exercise. These distinct responses were not attributable to differences in hypertrophy. In part II, in PH + shunt the contractility response was blunted compared with PH, which caused pseudonormalization of parameters. Additional volume overload strongly enhanced hypertrophy in PH. We conclude that different types of loading result in distinct patterns of RV adaptation. This is of importance for the approach to patients with chronically increased RV load and for experimental studies in various types of RV failure.

Right ventricular failure; contractility; pressure-volume analysis; pulmonary hypertension; monocrotaline

Right ventricular failure is a detrimental condition that is associated with significant morbidity and mortality in patients with congenital heart disease and/or pulmonary hypertension (PH) (11, 19, 34, 38). In these conditions, persistent abnormal loading of the right ventricle (RV) leads to RV failure in the long term (17, 50). However, physiological and molecular mechanisms of RV adaptation to these abnormal loading conditions and its derailment into RV failure are largely unknown (3, 50). As a consequence, no heart failure therapy exists that specifically targets the RV.

Different animal models have been used to study the abnormally loaded RV, but interpretation of data and translation to the clinical setting are hampered by conceptual concerns.

First, distinct types of RV overload are used in experimental models. These include the induction of PH, where the RV interacts with an increased dynamic load due to a high-resistance pulmonary vascular bed (i.e., peripheral-type pressure overload), and pulmonary arterial banding (PAB), resulting in an increased load with an absolute, fixed uncoupling of the ventriculo-vascular interplay (i.e., proximal-type pressure overload). It is unknown whether these distinct types of pressure overload result in common or distinct adaptive responses.

Clinical observations suggest that the RV tolerates congenital pulmonary valve stenosis (a proximal-type pressure overload) better than PH (a peripheral-type pressure overload) (24). This notion is supported by experimental data (6) and requires comparison of functional adaptation of the RV in models of either type.

Second, it is unknown how additional increased volume overload impacts the ability of the RV to adapt to increased pressure overload. This situation is clinically encountered in patients with congenital heart disease associated with systemic-to-pulmonary shunts who develop PH and appear to have a very poor prognosis (32).

Finally, most animal studies lack a “clinical” indicator of the severity of RV dysfunction and provide limited characterization of RV function, which complicates translation to the clinical setting.

We therefore aimed to characterize the clinical and functional RV response to distinct types of abnormal RV loading using voluntary exercise measurement and pressure-volume analysis. We focused on two clinically relevant perspectives: 1) proximal-type vs. peripheral-type RV pressure overload (PAB vs. PH), and 2) isolated peripheral-type pressure overload vs. peripheral-type pressure overload combined with volume overload (PH vs. PH + shunt).

We hypothesized that the response patterns of the RV, in terms of hemodynamics, clinical symptoms, and hypertrophy, would depend on the type of abnormal loading. This would have major consequences for both the interpretation of experimental research and its translation to clinical practice.

MATERIALS AND METHODS

Animal Models

Animal care and experiments were conducted according to the Dutch Animal Experimental Act; the investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996). The Animal
Experiments Committee of the University of Groningen, the Netherlands, approved the experimental protocol. The rats were individually housed with a 12:12-h light-dark cycle and fed ad libitum.

Ninety-four Wistar rats (male; 160–230 g; Harlan, Horst, the Netherlands) were assigned to one of the following experimental groups: 1) proximal-type pressure load via pulmonary artery banding (PAB, n = 10), 2) peripheral-type pressure load via monocrotaline-induced PH (PH, n = 16 and n = 17, low and high dose, see below), 3) PH combined with volume overload (PH + shunt, n = 14), 4) volume overload only (shunt, n = 10), and 5 and 6) sham-operated controls (CON, n = 9 and n = 18). Since not all experiments could be performed concurrently two separate control groups were created. Because of subtle differences in baseline hemodynamics between both control groups, relative changes of the abnormal loading groups (versus the corresponding control group) were compared, rather than absolute values for hemodynamic parameters. Number of rats used per analysis is also indicated in the figure legends.

PAB procedure was as described previously (7). PAB was performed via a left lateral thoracotomy, using an 18-gauge needle to standardize the degree of stenosis. For PAB surgery, rats were anesthetized with isoflurane/air mixture (5% for induction, 2–3% maintenance); analgesia was ensured by buprenorphine (0.01–0.05 mg/kg sc during surgery and the two following days).

PH was induced via subcutaneous injection of monocrotaline (MCT) (Sigma-Aldrich Chemie, Zwijndrecht, the Netherlands). To create a group of rats with a range of PH pressures, we used both monocrotaline 30 mg/kg body wt and 80 mg/kg body wt (see below). A monocrotaline dosage of 60 mg/kg body wt was used in the PH + shunt group because 80 mg/kg with additional aortocaval shunt led to rapid deterioration of the rats (<2 wk) and did not allow comparison to PH at a relevant time point.

Aortocaval shunt surgery was performed via a laparotomy as described previously, where standardization of the shunt size was reached by using an 18-gauge needle (4, 7, 13, 48). For shunt surgery rats were anesthetized with isoflurane/air mixture (5% for induction, 2–3% maintenance); analgesia was ensured by buprenorphine (0.01–0.05 mg/kg sc during surgery and the two following days).

The sham-operated control groups received sham-surgeries (thoracotomy and laparotomy) to control for PAB and aortocaval shunt procedures or saline-injection and laparotomy to control for monocrotaline injection and aortocaval shunt procedure.

Two animals (1 in shunt, 1 in a control group) died in the first week after surgery due to abdominal complications of the laparotomy. Additionally, two animals in the PH + shunt group died prematurely during echocardiography (before pressure-volume measurements could be performed). Their echocardiograms showed very poor RV function.

We made two comparisons in this study. Part I of this study compared peripheral-type pressure overload (PH) to proximal-type pressure overload (PAB) using 3 groups: peripheral-type pressure load (PH, monocrotaline 80 mg/kg body wt sc); proximal-type pressure load (PAB); and a sham-operated control group. To obtain a range of pressures, an additional group was treated with less monocrotaline (30 mg/kg body wt); data obtained in this group were added to the PH group only in linear regression analysis. Part II of the study compared isolated peripheral-type pressure overload to peripheral-type pressure overload combined with volume overload, and for this purpose four groups were used: PH (same group as in part I); PH + shunt (monocrotaline 60 mg/kg body wt + surgical aortocaval shunt); and a sham-operated control group. A group with isolated volume overload (aortocaval shunt) was used as additional control.

For a timeline of all measurements as well as an overview of the groups, we refer to Fig. 1.

Voluntary Exercise Measurements and Signs of Failure

To measure voluntary exercise (7), running wheels were mounted in the rat cages. Because of the large interindividual variation, rats were measured before and 4 wk after surgery/monocrotaline injection. Five days before surgery and 5 days before euthanasia, animals were allowed to run in the cage wheel. Running distance and time spent in the wheel were recorded daily using a digital magnetic counter (Commodoor Cycle Odometer, Commodoor, the Netherlands). The change in running distance at 4 wk vs. baseline was used as primary parameter of exercise.

Throughout the experiment, rats were daily examined for clinical signs of right ventricular failure. Clinical signs of heart failure were defined according to previously described “ABCDE-criteria” (7): A, appearance and activity; B, body weight; C, cyanosis and circulation; D, dyspnea and tachypnea; and E, edema and effusion.

Right Ventricular Hemodynamics

Hemodynamic characterization of the RV was performed by pressure-volume studies, obtained by right heart catheterization 4 wk after surgery as described previously (7). Rats were anesthetized with isoflurane (5% induction; 2–3% maintenance), intubated, and ventilated. The right jugular vein was dissected and cannulated facilitating hypertonic saline infusions. Following bilateral thoracotomy and pericardiotomy a combined pressure-conductance catheter (SPR-869, Millar Instruments, Houston, TX) was introduced via the apex into the RV and positioned in the RV outflow tract. RV pressures and conductance were recorded using a MPVS 400 processor at a sample rate of 1.000 Hz with Chart 5 (Millar Instruments). Analyses were performed offline using custom-made software (CircLab 2009/2010, P. Steendijk). The volume signal of the conductance catheter was calibrated for parallel conductance and slope factor (alpha) in order to obtain absolute volumetric values. The parallel conductance was estimated by infusing 10 μl of hypertonic (10%) saline via the jugular vein cannula (2). Slope factor was calculated as uncalibrated conductance catheter cardiac output divided by LV cardiac output, measured by echocardiography. Heart rate, pressures, maximal and minimal speed of pressure change (dP/dtmax and dP/dtmin), volumes, and relaxation constant tau were derived from steady-state measurements. End-systolic pressure-volume relations (ESPVVR), end-diastolic pressure-volume relations (EDPVR), and preload recruitable stroke work (PRSW) were determined from measurements obtained during transient progressive constriction of the vena cava inferior. The slopes of
Peak P, mmHg 26
RV hemodynamics in CON, PH, and PAB

Table 1. made using steady-state measurements from the individual animals. Volume-end-diastolic volume diagrams of the different groups were found consistently linear, not monoexponential, EDPVRs. Stroke stood as stiffness) was used as a measure of diastolic function: we the slope of EDPVR (end-diastolic elastance, which can be under-
ESPVR and PRSW were used as measures of systolic function, and the slope of EDPVR (end-diastolic elastance, which can be understood as stiffness) was used as a measure of diastolic function: we found consistently linear, not monoexponential, EDPVRs. Stroke volume-end-diastolic volume diagrams of the different groups were made using steady-state measurements from the individual animals.

Echocardiography

Echocardiography was performed on the day before animals were euthanized as described before (7). Rats were anesthetized with isoflurane (5% induction; 2–3% maintenance). Echocardiography was performed using a Vivid Dimension 7 system and 10S-transducer (GE

Table 1. RV hemodynamics in CON, PH, and PAB

<table>
<thead>
<tr>
<th></th>
<th>CON 1</th>
<th>CON 2</th>
<th>PH</th>
<th>PAB</th>
<th>CON PH</th>
<th>CON PAB</th>
<th>PH PAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak P, mmHg</td>
<td>26 ± 2</td>
<td>25 ± 1</td>
<td>50 ± 4</td>
<td>96 ± 16</td>
<td>70 ± 9</td>
<td>144 ± 24</td>
<td>&lt;0.001 0.001 0.041</td>
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<td>Wall stress peak, mmHg</td>
<td>156 ± 11</td>
<td>151 ± 5</td>
<td>277 ± 26</td>
<td>83 ± 17</td>
<td>492 ± 62</td>
<td>214 ± 40</td>
<td>0.015 &lt;0.001 0.001</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>1 ± 0.3</td>
<td>2 ± 0.6</td>
<td>5 ± 1</td>
<td>199 ± 62</td>
<td>6 ± 1</td>
<td>428 ± 119</td>
<td>0.045 0.001 0.034</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>306 ± 10</td>
<td>351 ± 14</td>
<td>326 ± 16</td>
<td>5 ± 1</td>
<td>300 ± 10</td>
<td>2 ± 3</td>
<td>0.21 0.78 0.37</td>
</tr>
<tr>
<td>SV, µl</td>
<td>269 ± 7</td>
<td>290 ± 15</td>
<td>275 ± 20</td>
<td>5 ± 7</td>
<td>227 ± 19</td>
<td>16 ± 7</td>
<td>0.54 0.09 0.22</td>
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<td>CI, ml/min·kg BW−1</td>
<td>0.26 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>8 ± 5</td>
<td>0.24 ± 0.02</td>
<td>5 ± 8</td>
<td>0.25 0.53 0.68</td>
</tr>
<tr>
<td>ESV, µl</td>
<td>320 ± 42</td>
<td>287 ± 41</td>
<td>431 ± 41</td>
<td>50 ± 14</td>
<td>648 ± 29</td>
<td>102 ± 9</td>
<td>0.010 &lt;0.001 0.012</td>
</tr>
<tr>
<td>EDV, µl</td>
<td>589 ± 47</td>
<td>577 ± 46</td>
<td>706 ± 57</td>
<td>30 ± 7</td>
<td>874 ± 39</td>
<td>48 ± 6</td>
<td>0.10 0.001 0.032</td>
</tr>
<tr>
<td>EF, %</td>
<td>47 ± 3</td>
<td>52 ± 4</td>
<td>40 ± 1</td>
<td>25 ± 2</td>
<td>26 ± 1</td>
<td>45 ± 3</td>
<td>&lt;0.001 0.002 0.010</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>1.315 ± 75</td>
<td>1.634 ± 114</td>
<td>2.030 ± 194</td>
<td>24 ± 12</td>
<td>2.812 ± 471</td>
<td>114 ± 36</td>
<td>0.32 &lt;0.001 0.0002</td>
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<tr>
<td>dP/dtmax – min%Hg/s</td>
<td>964 ± 41</td>
<td>1,322 ± 127</td>
<td>1,976 ± 155</td>
<td>50 ± 12</td>
<td>2,491 ± 256</td>
<td>158 ± 27</td>
<td>0.018 &lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>dP/dtmax ind, 1/s</td>
<td>51 ± 3</td>
<td>61 ± 2</td>
<td>42 ± 2</td>
<td>32 ± 3</td>
<td>40 ± 3</td>
<td>21 ± 6</td>
<td>&lt;0.001 0.007 0.09</td>
</tr>
<tr>
<td>dP/dtmax ind, −1/s</td>
<td>37 ± 1</td>
<td>48 ± 1</td>
<td>41 ± 3</td>
<td>13 ± 7</td>
<td>38 ± 3</td>
<td>1 ± 9</td>
<td>0.14 0.92 0.14</td>
</tr>
<tr>
<td>Ees, mmHg/ml</td>
<td>59 ± 8</td>
<td>47 ± 6</td>
<td>134 ± 40</td>
<td>188 ± 87</td>
<td>155 ± 27</td>
<td>164 ± 46</td>
<td>0.035 0.049 0.78</td>
</tr>
<tr>
<td>PRSW, mmHg</td>
<td>12 ± 2</td>
<td>24 ± 4</td>
<td>37 ± 8</td>
<td>53 ± 32</td>
<td>44 ± 6</td>
<td>283 ± 54</td>
<td>0.039 &lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>15.6 ± 1.1</td>
<td>12.5 ± 0.5</td>
<td>16.4 ± 1.0</td>
<td>31 ± 8</td>
<td>20.7 ± 1.4</td>
<td>33 ± 9</td>
<td>0.011 0.014 0.86</td>
</tr>
<tr>
<td>Tau corr, ms</td>
<td>78 ± 4</td>
<td>73 ± 3</td>
<td>88 ± 8</td>
<td>21 ± 11</td>
<td>103 ± 4</td>
<td>31 ± 6</td>
<td>0.10 0.032 0.45</td>
</tr>
<tr>
<td>Ees, mmHg/ml</td>
<td>3.6 ± 0.9</td>
<td>5.4 ± 0.7</td>
<td>5.6 ± 1.2</td>
<td>4 ± 23</td>
<td>9.3 ± 1.8</td>
<td>158 ± 50</td>
<td>0.92 0.004 0.003</td>
</tr>
</tbody>
</table>

Data are means ± SE. Abs are absolute values; Rel Δ values are percentage change vs. respective control group. Peak P, peak pressure, EDP, end-diastolic pressure, HR, heart rate, SV, stroke volume, CI, cardiac index, ESV, end-systolic volume; EDV, end-diastolic volume; EF, ejection fraction; dP/dtmax ind and dP/dtmax and are indexed for peak pressure (max) and end-diastolic pressure (min), respectively; Ees, end-systolic elastance, PRSW, preload recruitable stroke work; tau, relaxation constant; tau corr, tau corrected for heart rate; Eed, end-diastolic elastance; CON, control; PH, pulmonary hypertension; PAB, pulmonary artery banding. All hemodynamic parameters were obtained from pressure-volume analysis, except cardiac output, which was measured by echocardiography. Significance indicated by P values.
Healthcare, Waukesha, WI). Systolic aorta diameter was measured in parasternal long-axis view (in triplo): pulsed-wave Doppler of aorta flow was obtained in the five-chamber view. Stroke volume was calculated as (aorta diameter)$^2 \times 3.14 \times$ velocity time integral (VTI). To obtain cardiac output, stroke volume was multiplied by the heart rate, calculated from the duration of the heart beat in the Doppler signal. The mean of measurements from 6–12 consecutive beats with a proper signal was taken to average out beat-to-beat variation.

Termination, Organ Weights, and Hypertrophy

After heart catheterization, the rats were terminated by removing the heart from the thorax. Heart, lungs, and liver were dissected. RV, interventricular septum, left ventricle, and atria were separated and weighed.

qRT-PCR, Western Blot, Immunohistochemistry

To further analyze the remodeling response of the RV, we performed qRT-PCR, Western blotting, and immunohistochemistry. For assessment of the activity of key regulating pathways of hypertrophy, we measured Erk1/2 (MAP kinase pathway), Akt (PI3K pathway) and regulator of calcineurin type 1 (RCAN1; calcineurin-NFAT pathway). To see whether distinct loading conditions elicited differences in fetal reprogramming we measured natriuretic propeptide type A (NPPA) and myosin heavy chain isofoms beta and alpha (MYH7 and 6).

Western blotting. Protein was extracted from snap-frozen RV tissue using RIPA buffer. We used antibodies to phosphorylated and total Akt (1:100; Cell Signalling Technology, Danvers, MA) and phosphorylated and total MAP kinase ERK1/2 (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) (47). Primary antibody binding was visualized by horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Perkin-Elmer, Waltham, MA).

Immunohistochemistry. Transversal midventricular RV tissue-slices were fixated using 4% formalin and embedded in parafin. Four-micrometer-thick sections were cut, deparaffinized, and rehydrated in decreasing graded alcohol and xylene. For determination of cardiomyocyte surface area, these were stained using Gomori’s reticulin silver staining and photographed using a camera fitted on a microscope (Zeiss Benelux, Sliedrecht, the Netherlands) at 40× magnification and analyzed using Image Pro software (MediaCybernetics, Bethesda, MD). Only transversally cut myocytes were included; per section measurements were averaged from 60 cells in 4 different fields.

Fibrosis, PDE5A-Axis, and PKG-1 Activity

For determination of the amount of fibrosis, RV sections were stained using NovaUltra Masson Trichrome Stain kit (HCI World, Woodstock, MD) and photographed using a digital slide scanner (NanoZoomer 2.0-HT, Hamamatsu Photonics Netherlands, Aldermere, the Netherlands) at 20× magnification and analyzed using Image Scope 11 (Aperio Technologies, Vista, CA). The extent of fibrosis was quantified as the blue-stained percentage of the total tissue area, measured per whole section. The edges of the tissue and major vessels including perivascular fibrosis were excluded from analysis to obtain purely myocardial interstitial fibrosis. The phosphodiesterase type 5A-protein kinase G1 (PDE5A-PKG1) axis is suggested to be involved in the RV response to abnormal loading (37) and might have specific relevance to diastolic function (5, 27). We analyzed the activity of this pathway by measuring PDE5A mRNA and protein expression, myocardial cGMP levels, and PKG-1 activity as described previously (7, 37).

Statistical Analysis

Quantitative data are expressed as means ± standard error of the mean (SE) for the control group and percentage change versus matched control group for all other groups, unless mentioned otherwise. All variables were tested for normality. Differences between groups were evaluated using one-way ANOVA followed by post hoc analysis (protected Fisher’s least significant difference method) or Kruskal-Wallis and Mann-Whitney U-test, as appropriate. To examine the interaction between RV pressure and various parameters and evaluate differences between groups we performed linear regressions and two-way ANOVA. P < 0.05 was considered significant (PASW Statistics 18 for Windows, SPSS, Chicago, IL).

RESULTS

PH and PAB elicit distinct hemodynamic responses in the RV

The RV responded to pressure overload with enhancing contractility (measured by heart catheterization; representative

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Fig. 3. Voluntary exercise and RV failure symptoms in PH and PAB. Exercise vs. RV peak pressure. Exercise is expressed as relative change in distance run before animals were euthanized vs. baseline (n = 6–15). Means ± SE for the control group and percentage change versus matched control group for all other groups, unless mentioned otherwise. Significance is vs. CON. B: percentage of rats presenting clinical signs of RV failure (n = 9–16). A, B, C, D, E refer to symptom categories: A, activity and appearance; B, body weight; C, cyanosis and/or hampered peripheral circulation; D, dyspnea and/or tachypnea; E, effusions: pleural or ascites.
PV loops in Fig. 2A): end-systolic elastance (Ees) and preload recruitable stroke work (PRSW) were significantly increased in both PH and PAB (Fig. 2B). However, the degree of increase was lower in PH. In PH, PRSW was correlated to peak pressure (Fig. 2C), in contrast to PAB (Fig. 2C). Ees was not correlated with increasing pressure in either of the models.

Diastolic function (as measured by heart catheterization) deteriorated in PAB, but not in PH. End-diastolic elastance (Eed, Fig. 2D) and relaxation constant tau (Table 1) were increased in PAB, but not in PH. Active myocardial relaxation was estimated to be complete at 3.5 times uncorrected tau \( \approx 73 \) ms (52). The total duration of diastole was \( \approx 100–140 \) ms at
Table 2. RV hemodynamics in CON, PH + shunt, PH, and shunt

<table>
<thead>
<tr>
<th></th>
<th>CON 1</th>
<th>CON 2</th>
<th>Abs</th>
<th>Rel Δ, %</th>
<th>PH + Shunt</th>
<th>CON 2</th>
<th>Abs</th>
<th>Rel Δ, %</th>
<th>Shunt</th>
<th>Abs</th>
<th>Rel Δ, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak P, mmHg</td>
<td>26 ± 2</td>
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<td>Wall stress peak, mmHg</td>
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<td>277 ± 26</td>
<td>83 ± 17</td>
<td>241 ± 18</td>
<td>59 ± 12</td>
<td>336 ± 39</td>
<td>114 ± 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>1 ± 0.3</td>
<td>2 ± 0.6</td>
<td>5 ± 1</td>
<td>199 ± 62</td>
<td>5 ± 0.6</td>
<td>182 ± 31</td>
<td>2 ± 0.8</td>
<td>114 ± 74</td>
<td></td>
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</tr>
<tr>
<td>HR, beats/min</td>
<td>306 ± 10</td>
<td>351 ± 14</td>
<td>326 ± 16</td>
<td>−5 ± 1</td>
<td>288 ± 14</td>
<td>−21 ± 2</td>
<td>304 ± 7</td>
<td>−4 ± 8</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SV, μl</td>
<td>269 ± 7</td>
<td>290 ± 15</td>
<td>275 ± 20</td>
<td>−5 ± 7</td>
<td>480 ± 31</td>
<td>66 ± 11</td>
<td>415 ± 24</td>
<td>55 ± 9</td>
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<tr>
<td>Cl, ml·min⁻¹·g BW⁻¹</td>
<td>0.26 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>−8 ± 5</td>
<td>0.44 ± 0.04</td>
<td>57 ± 14</td>
<td>0.30 ± 0.02</td>
<td>52 ± 9</td>
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<tr>
<td>ESV, μl</td>
<td>320 ± 42</td>
<td>287 ± 41</td>
<td>431 ± 41</td>
<td>50 ± 14</td>
<td>652 ± 149</td>
<td>127 ± 52</td>
<td>839 ± 49</td>
<td>162 ± 15</td>
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<tr>
<td>EDV, μl</td>
<td>589 ± 47</td>
<td>577 ± 46</td>
<td>706 ± 57</td>
<td>30 ± 7</td>
<td>989 ± 62</td>
<td>71 ± 11</td>
<td>1,257 ± 61</td>
<td>113 ± 10</td>
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<td>EF, %</td>
<td>47 ± 3</td>
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<td>dP/dt max, mmHg/s</td>
<td>1,315 ± 75</td>
<td>1,634 ± 114</td>
<td>2,030 ± 194</td>
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<td>2,470 ± 125</td>
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<td>dP/dt max, −mmHg/s</td>
<td>964 ± 41</td>
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<tr>
<td>Ees, mmHg/ml</td>
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<td>134 ± 40</td>
<td>188 ± 87</td>
<td>84 ± 30</td>
<td>81 ± 64</td>
<td>41 ± 4</td>
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<td>25 ± 3</td>
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<td>Tau corr, ms/s</td>
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<td>73 ± 3</td>
<td>88 ± 8</td>
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<td>72 ± 9</td>
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<td>4 ± 23</td>
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<td>3.9 ± 1.6</td>
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P Values

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<th>Shunt vs. CON</th>
<th>PH vs. PH</th>
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Data are means ± SE. Abs are absolute values; Rel Δ values are percentage change vs. respective control group. Shunt, aorticaval shunt; PH + shunt, PH + aortocaval shunt. All hemodynamic parameters were obtained from pressure-volume analysis, except cardiac output, which was measured by echocardiography. Significance indicated by P values.

Heart rate 300 beats/min (0.5–0.66 × heart beat duration), so increased end-diastolic elastance did not reflect incomplete relaxation, but increased stiffness.

 Ventricular dilatation and a rightward shift of the Frank-Starling curve (derived from heart catheterization data) were present in both groups. The latter was more prominent in PAB than in PH (Fig. 2E), indicating that, at a similar preload, output was lower in PAB than in PH.

 Voluntary exercise and clinical signs of RVF differ between PH and PAB. PAB reduced voluntary exercise and induced clinical signs of RV failure, such as dyspnea and edema, more than PH (Fig. 3, A and B). The severity of reduction in exercise was not related to RV peak pressure, especially beyond 30% increase of normal RV peak pressure (Fig. 3A).

 Hypertrophy and regulators are equally induced in PH and PAB. Pressure load induced RV hypertrophy (Fig. 4A), regardless of whether it resulted from PH or PAB (Fig. 4B), and RV weight correlated with peak pressure (Fig. 4C). Upregulation of RCAN1 (Fig. 4D) and MHC-isotype switch (Fig. 4E) was also correlated with peak pressure equally in both PH and PAB, although two PAB animals lacked a strong MHC-isotype switch (arrows in Fig. 4E). There was no significant fibrosis, and protein expression and phosphorylation status of Akt and ERK1/2 were unchanged in both types of RV pressure overload (data not shown). PDE5A expression (mRNA and protein) was at control level in both PH and PAB, but PKG-1 activity tended to be higher in PH (2.83 ± 0.37 AU) than in PAB (1.87 ± 0.26 AU, P = 0.07 vs. PH) and CON (1.95 ± 0.13 AU, P = 0.11 vs. PH).

II. Pulmonary Hypertension vs. Pulmonary Hypertension + Shunt

Combined overload blunts the contractility response, pseudonormalizing hemodynamic parameters. Pressure-volume analysis showed that the addition of volume overload to
peripheral-type pressure overload induced further RV dilatation (Fig. 5A) and resulted in compromised contractility compared with PH (Fig. 5B). Ees did not increase in response to isolated volume load, whereas PRSW did.

The change in PRSW following pressure load depended on the type of load (Fig. 5C): in PH + shunt, PRSW barely increases in response to pressure load, compared with PH or shunt.

PH + shunt had a tendency toward diastolic dysfunction (increased end-diastolic elastance), but this did not reach significance due to large interanimal variation. Also the other invasively measured parameters of diastolic function did not indicate pronounced diastolic dysfunction (Table 2). Further, PH + shunt showed a leftward shift of the Frank-Starling curve (Fig. 5E). This, however, may reflect the reduced heart rate found in these animals, rather than augmented stroke volume in response to increased preload (Table 2).

**Reduced voluntary exercise and prominent clinical signs of RVF in combined overload.** Voluntary exercise was significantly reduced in both PH + shunt and shunt compared with CON (Fig. 6A). However, the majority of rats with PH + shunt exhibited signs of RVF, in contrast to those with PH or shunt (Fig. 6B).

**Combined overload induces additional hypertrophy and gene expression.** PH + shunt induced more RV hypertrophy than PH or shunt (Fig. 7A). The very prominent RV hypertrophy in PH + shunt seems an addition of pressure- and volume-induced hypertrophy as the RV weights in these rats equaled the sum of increases of RV weight in shunt and PH (Fig. 7B).

Combined overload also had a strong additive effect on MYH-isoform switch (Fig. 7C) and NPPA expression (Fig. 7D), whereas RCAN1 upregulation was similar in PH, PH + shunt, and shunt (Fig. 7E). Protein expression and phosphorylation status of Akt and ERK1/2 were unchanged in these groups (data not shown). In both the combined overload and isolated volume overload there was no significant fibrosis. PDE5A expression (mRNA and protein) was unchanged (data not shown). Also, PKG-1 activity was similar in PH, PH + shunt, and shunt (data not shown).

**DISCUSSION**

This study characterizes right ventricular responses to distinct types of chronic RV overload and demonstrates that the pattern of response depends on the type of loading. We found experimental proximal-type pressure load (PAB) to induce significant diastolic dysfunction after 4 wk, whereas peripheral-type pressure load (PH) did not, independent of severity of RV hypertrophy. Also, PAB was associated with more prominent RV dilatation and clinical signs of RV failure compared with PH. The combination of volume load and PH resulted in additional RV hypertrophy and a pattern of pseudonormalization with blunted contractility response.

These different responses to distinct loading conditions imply that experimental studies on RV failure should use models that match the loading condition of the clinical disease under study. Moreover, it may be of clinical relevance in the therapeutic approach of patients with different types of RV loading.

**The RV Responds to Chronic Pressure Overload with Both Frank-Starling Mechanism and Increased Contractility**

In chronic pressure overload, the left ventricle is thought to first utilize the Frank-Starling mechanism, increasing preload to maintain output, and once its limit has been reached, to increase contractility to compensate for the “afterload mismatch” (42). This concept has been confirmed in aortic banding models (9, 36), but it is unclear whether it is applicable to the RV. Previous studies, both experimental and clinical, suggested that in the proximal-type pressure overload, increased contractility rather than Frank-Starling mechanism characterizes the RV response (15, 30), whereas in the peripheral-type pressure overload, the RV response is limited to the Frank-Starling mechanism and does not increase contractility (21). By direct comparison, however, this study shows that in both proximal- and peripheral-type pressure overload the RV response uses the Frank-Starling mechanism, but additionally depends on increased contractility for maintaining stroke volume. The LV concept of adaptation to chronic pressure overload therefore seems to hold also in the RV. However, the proximal-type pressure overload appears to force the RV to function at the limit of its adaptive capacity more, or earlier, than peripheral-type pressure overload, illustrated by the more pronounced right shift of the Frank-Starling curve and higher Ees and PRSW in PAB.

**Additional Volume Overload in PH Blunts the Contractility Response, Pseudonormalizing Hemodynamic Parameters**

Recognizing the importance of preload for the pressure-overloaded RV, we investigated the effect of additional volume...
overload, which might be beneficial through maintaining stroke volume via the Frank-Starling mechanism. In the present study the RV indeed reached higher stroke volumes with additional volume overload. However, in contrast to isolated pressure overload, contractility failed to increase. This is in line with reports that the chronically volume-overloaded RV cannot increase contractility in response to acute pressure overload (46). Studies in large animal models of combined volume and pressure overload have shown that during disease progression contractility initially increases, but falls back to pseudonormal levels in a more advanced stage of RV dysfunction (28, 40, 41). Causal factors for the blunted contractility response may include loss of peristaltic contraction pattern (33), disturbed calcium homeostasis (8, 26), and changes in coronary perfusion (46). Failure to increase contractility has also been reported in a model of chronic LV volume overload (25). Also, in LV models, pressure load (aortic banding) and volume load (aortocaval shunt) have been shown to induce distinct functional and molecular responses [e.g., in hypertrophy signaling and calcium handling (35, 47)]. Combined pressure-volume load may then lead to adverse, maladaptive remodeling. Excessive hypertrophy with a strong switch toward the slow-type \( /H9252 \)-myosin heavy chain, associated with poor contractile function (44), found in the present study, might also play a causal role. The blunted contractility response, causing pseudonormalization of Ees and PRSW, carries the risk of underestimating RV dysfunction in patients with combined overload of the RV.

**Diastolic Function in PAB, PH, and PH + Shunt**

Another important observation in this study was the diastolic dysfunction present in PAB but not in PH rats. Although diastolic dysfunction in PAB has been previously and consistently reported (7, 18, 30), data in experimental PH were contradictory (21, 29). The absence of evident diastolic dysfunction in PH in our study is rather convincingly demonstrated by using a load-independent parameter (end-diastolic elastance), confirmed by secondary parameters such as relaxation constant tau, maximum speed of pressure decline \( dP/dt_{\text{min}} \), and end-diastolic pressure. Nevertheless, PH is thought to also cause diastolic dysfunction, and we propose our data suggest an intrinsic difference in RV adaptation between PAB and PH.

In the present study, PAB after 4 wk led to significantly disturbed diastolic function accompanied by poor exercise and a high proportion of clinical signs of RVF. Although the increased end-diastolic elastance resulted from increased stiffness (and not incomplete relaxation), these differences were not accounted for by hypertrophy, which was comparable in both groups, or by myocardial fibrosis, which had not yet developed at this time point (7, 21).

The prolonged active relaxation (tau) and increased passive stiffness (end-diastolic elastance) observed closely resemble the diastolic disturbances seen in left-sided diastolic heart failure (54), where protein kinase G-1 (PKG-1) activity has been shown to be impaired (49). By phosphorylating specific domains of the sarcomeric protein titin (27), PKG-1 may improve diastolic properties (5). Indeed, we found a trend of increased PKG-1 activity in PH, but not in PAB, indicating a potential protective mechanism against diastolic dysfunction. As the RV might be particularly vulnerable to diastolic dysfunction (10), these findings identify new targets for further study on causative mechanisms of RV failure. These differences in PKG-1 activity suggest that adaptation mechanisms to
proximal vs. peripheral pressure overload are different. PKG-1 activity also differs in distinct forms of LV disease (49), but its exact role in proximal vs. peripheral LV pressure overload remains to be elucidated. In general both common and distinct adaptive mechanisms and differences in vulnerability for diastolic dysfunction have been suggested in models of LV peripheral pressure load (systemic hypertension) vs. proximal pressure load (aortic banding) (reviewed in 14, 22). However, as was the case for the RV, direct comparisons are lacking. Therefore, when comparing the various studies, it is difficult to rule out confounding effects of time course, model inductors [e.g. streptozotocin (1, 16)] or inherent differences in genetic make-up in different strains [e.g., in spontaneously hypertensive rats (31, 45, 53)].

In PH + shunt, no diastolic dysfunction could be demonstrated despite apparent clinical signs in the majority of animals, including enlarged right atrium, pleural effusion, and ascites. Diastolic dysfunction might have been masked by the significantly lower heart rates found in this group.

Voluntary Exercise in Models of Abnormal RV Loading

Although experimental data cannot be directly translated to clinical patients, the use of clinically relevant parameters in animal models may be of value. In this study, voluntary exercise measurement was used because of its analogy with the 6-min walk test, a submaximal exercise test clinically used in PH patients to assess functional capacity and efficacy of treatment (43). Although the optimal definition of clinical heart or RV failure is still debated (20), parameters of functional capacity are usually involved. In general, studies on experimental RV adaptation to abnormal loading and RV failure have not provided such data. We propose exercise data are a valuable adjunct to the interpretation and clinical relevance of ventricular functional parameters usually measured in rest conditions. This study demonstrates that different RV response patterns associated with distinct types of overload were indeed reflected in differential decreases in voluntary exercise.

Limitations

The use of anesthesia may affect cardiac function but is inevitable in animal studies using pressure-volume measurements. Since we used isoflurane, shown to have only mild negative effects on inotropy and cardiac index in rodents (23) and since the groups compared were all subjected to identical regimens of anesthesia, we contend that this did not limit the answers to our research questions.

We calibrated for slope factor α using echo-measured cardiac output, which is performed with closed chest, while the catheterization is performed with open chest. This might have led to a consistent overestimation of the cardiac output and, thus, of ventricular volumes. However, the observed ventricular volumes in this study are in line with previous reports (21, 39). Moreover, the concomitant use of relative increases in volumes (which is not affected by this overestimation) discarded this potential limitation.

Assessment of the temporal development of RV responses was not feasible in our set-up, but would provide additional valuable data and is a worthwhile objective for future studies.

When studying pathophysiological mechanisms in an animal model, ideally the model should be representative for the human disease that it mimics (12). This means that for the pressure- and/or volume-loaded RV it ideally should be characterized by 1) triggers recognized in human disease (e.g., altered pulmonary blood flow) and 2) a clinical course of progressive RV dysfunction leading to death. We therefore used pressure load (through PAB) and volume load (through aortocaval shunt) as clinically recognized inducers of progressive RV failure. In volume load, we used an additional trigger (monocrotaline) to induce progressive pulmonary vascular neointimal lesions to further mimic the human conditions seen in pulmonary arterial hypertension associated with congenital heart disease (13). It should be noted that monocrotaline has been suggested to have direct effects on the RV. Such effects might have contributed to the observed RV responses to monocrotaline-induced peripheral pressure overload. Further, the aortocaval shunt results in biventricular volume overload. Some of the clinical parameters measured in shunt and PH + shunt could be influenced by LV volume load, but it has been shown that at 4 wk rats with an aortocaval shunt have normal LV function (51). Confirmation of the described results in other animal models of RV pressure overload, e.g., hypoxia (whether or not combined with the vascular endothelial growth factor receptor blocker Sugen 5416) or fawn-hooded rats, may indicate to what extent the conclusions from this study could be generalized, although these models represent less recognizable clinical triggers for human RV failure.

As in all preclinical studies, findings in rodent models must not be overinterpreted and must be used with caution when translating such findings to human patients.

Conclusion

This study sheds new light on the RV response to different types of abnormal loading. First, proximal-type pressure load (PAB) induced more diastolic dysfunction than peripheral-type pressure load (PH). Second, the combination of PH and volume load resulted in additional RV hypertrophy and a pattern of pseudonormalization with blunted contractility response. The distinct responses imply that experimental studies in various types of RV failure should use models that match the loading condition of the studied disease. Moreover, it may be of clinical relevance in the therapeutic approach of patients with different types of RV loading.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


