Right and left ventricular function after chronic pulmonary artery banding in rats assessed with biventricular pressure-volume loops

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Submitted 19 March 2006; accepted in final form 3 May 2006


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RIGHT VENTRICULAR (RV) function is an important determinant of long-term outcome in patients with complex congenital heart disease, chronic pulmonary obstructive diseases, or pulmonary hypertension. In many of these patients, the RV is subjected to (residual) abnormal loading conditions, including pressure overload. Although compensated hypertrophy will develop initially, ultimately RV failure will ensue. The mechanisms underlying the progression from compensated RV hypertrophy to decompensated RV hypertrophy (i.e., RV failure) have not been well defined. As the survival of the patients improves, a better understanding of these mechanisms becomes mandatory to be able to design preventive strategies and to time surgical (re)intervention in these patients.

To study the mechanisms underlying the transition from a compensated state of hypertrophy to a decompensated state in patients is very difficult, because invasive data cannot be easily obtained. For this purpose, animal models may be beneficial. Small experimental animals, such as rats, are widely used in cardiovascular research since they can provide a variety of disease models, including heart hypertrophy and failure. A major advantage of the use of these small disease models is that cardiac material can be easily sampled to study critically involved molecular changes over time and the possibility to study effects of transgenesis and gene ablation (2, 5, 22, 23, 34). Recently, we performed a proteomic profiling study on RV hypertrophy showing multiple expression and posttranslational changes in metabolic, stress, and myofilament proteins (12). To interpret these molecular findings in terms of possible mechanisms involved in RV remodeling, an accurate assessment of RV function is required, preferably by using load-independent parameters of cardiac contractility (13, 24, 29, 30). We present a biventricular hemodynamic characterization using pressure-volume (PV) loops of RV hypertrophy in a rat model induced by 6 wk of pressure overload as a result of pulmonary artery banding (PAB).

METHODS
All experimental procedures and protocols used in this investigation were reviewed and approved by the institutional animal care and use committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

Male Wistar rats (190–220 g; Harlan, Zeist, The Netherlands) underwent PAB or sham operation. Complete hemodynamic studies were performed in 15 animals: 6 rats underwent PAB at the age of 8 wk, whereas 9 rats underwent a sham operation and served as control. The animals were housed after the initial operation (i.e., sham or PAB) for a period of 6 wk, before the hemodynamic studies, in groups of two or three animals, on a 12:12-h light/dark cycle with standard rat chow and water ad libitum.

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PAB procedure. Anesthesia was induced by pentobarbital sodium (60 mg/kg ip). After intubation, the animals were mechanically ventilated with the use of a volume-controlled respirator and oxygen-enriched room air. Positive end-expiratory pressure was maintained at 4 cmH2O. A left thoracotomy was performed, and the pulmonary artery (PA) was carefully dissected free from the aorta. A silk thread was positioned under the PA, and an 18-gauge needle was placed alongside the PA. A suture was tied tightly around the needle, and the needle was rapidly removed to produce a fixed constricted opening in the lumen equal to the diameter of the needle. The combination of a fixed banding around the PA and the growth of the animal will eventually result in a markedly increased RV afterload. After the banding, the thorax was closed in layers, and postoperative pain relief was obtained by applying buprenorphine (15 μg/kg sc). The sham animals underwent the same procedure except for the banding of the PA.

Hemodynamic instrumentation. After a housing period of 6 wk, hemodynamic measurements were performed. The study protocol was the same for sham and PAB animals. After induction of anesthesia (60 mg/kg ip pentobarbital sodium), the animals were intubated and ventilated as described above. A catheter (PE-50) was placed in the abdominal cavity for intraperitoneal administration of pentobarbital sodium for maintenance of anesthesia. Both femoral veins were cannulated with a catheter (PE-50) for the infusion of hypertonic saline for calculation of the parallel conductance (see Conductance catheter calibration) and for the administration of dobutamine. The right carotid artery was cannulated with a catheter (PE-50) connected to a fluid-filled pressure transducer to monitor systemic blood pressures and to obtain samples for blood-gas analysis. The right jugular vein was cannulated with a catheter (PE-50) for the infusion of Hemaccel (Hoechst; Behring-Werke, Marburg Germany) to maintain adequate fluid levels. A right thoracotomy was performed and an ultrasonic flow probe (Transonic Systems, Ithaca, NY), connected to a Transonics flowmeter (TS420), was placed around the aorta and used for calibration of the conductance catheter (see Conductance catheter calibration). A left thoracotomy was performed for maximal exposure of the left side of the heart to facilitate an adequate catheter insertion in the left ventricle (LV). For preload reduction, required to obtain systolic and diastolic PV relations, a silk thread was placed around the vena cava inferior, just above the diaphragm. A conductance catheter (CD Leycom, Zoetermeer, The Netherlands) and a pressure-tip catheter (Millar Instruments, Houston, TX) were inserted in the RV through the venricular wall, at the level of the outflow tract, and positioned toward the apex. Similarly, conductance and pressure-tip catheters were inserted in the LV apex and positioned along the LV long axis. The conductance catheters consisted of five segments of which, on average, two to three segments were used for measurement of ventricular volumes. Positioning of the conductance catheters was optimized by observing the pressure and segmental volume signals with appropriate phase relationships. The conductance catheters were connected to Leycom Sigma-5 DF signal processors (CD Leycom), and the pressure-tip catheters were connected to pressure transducer units (Millar Instruments). Signals were recorded at a minimal sample rate of 500 Hz using the Conduct 2000 data-acquisition hardware and software (CD Leycom) installed on an IBM-compatible personal computer.

Conductance catheter calibration. To obtain absolute volumes, the conductance catheter-derived signals must be calibrated for parallel conductance and slope factor. Conductance catheters were calibrated as previously described. Briefly, parallel conductance was determined by the hypertonic saline method, and slope factor by matching uncalibrated conductance stroke volume with stroke volume derived from the aortic flow signal (1, 28).

Hemodynamic study protocol. When hemodynamic stability was reached, a set of measurements was performed to calibrate the conductance catheter method and to assess hemodynamics and contractile performance in baseline conditions. Data were recorded with open chest at steady-state baseline conditions and during transient preload reduction. The parallel conductance of both ventricles was measured (in duplicate) by injecting 10% of 50 μl NaCl intravenously (28). All measurements were made during short suspension of the ventilation at end expiration. To determine inotropic reserve, we infused dobutamine at 2.5 (dobo-2.5) and 5 (dobo-5) μg·kg−1·min−1 via a pump. The same set of measurements as described above was performed and started at least 10 min after the onset of dobutamine infusion. Moreover, the parallel conductance was recalculated for each dobutamine step, since parallel conductance changes during the administration of dobutamine (33). Before each set of measurements, a blood sample was drawn and analyzed (Roche Diagnostics, Almere, The Netherlands) to ensure proper oxygenation and acid-base balances.

Hemodynamic measurements and calculations. The biventricular signals were analyzed by custom-made software (Circlab). The steady-state data were averaged over two separate intervals that each consisted of at least five cardiac cycles. From these steady-state data, the following parameters were calculated: heart rate, cardiac output, stroke volume, end-systolic pressure, end-diastolic pressure, end-systolic volume, end-diastolic volume (Ved), maximal first time-derivative of pressure (dP/dtmax), and stroke work.

PV loops acquired during vena cava occlusion were used to derive ventricular PV relations (Fig. 1). The end-systolic point was defined as the point in the cardiac cycle of maximal elastance. Elastance was defined as P(t)/[V(t) − V0], where P(t) is the instantaneous pressure, V(t) instantaneous volume, and V0 the theoretical volume at zero pressure (8). V0 was determined by an iterative algorithm previously described by Kono et al. (16). The following relations were determined and used as parameters of systolic function: the end-systolic
Baseline hemodynamics. In baseline conditions, the heart rate and cardiac output did not differ between sham and PAB rats. The RV peak systolic pressures in PAB rats were increased to 60% of peak systolic LV pressure. RV end-diastolic and end-systolic volumes were slightly decreased in PAB rats, although this did not reach statistical significance ($P \geq 0.26$). Both RV stroke work and $dP/dt_{max}$ were increased in the PAB rats compared with the sham group. The slopes of the ESPVR, PRSW, and the $dP/dt_{max}$-$V_{ed}$ relations (henceforth called the three PV relations) were all steeper in the PAB rats, indicating an increased contractile function of the RV (Fig. 2 and Table 2).

In the LV, the end-systolic and end-diastolic pressures were similar in both groups. The $V_{ed}$ were similar for both groups, whereas the end-systolic volumes were decreased in the LV of the PAB rats. Both LV stroke work and $dP/dt_{max}$ were similar for both sham and PAB rats. The contractility of the LV, as expressed by the three PV relations, was not altered in the PAB rats.

RESULTS

During the banding period, the rats did not show overt signs of heart failure and/or cyanosis. The average body weight of the PAB group at the start of the protocol did not differ significantly from the control (sham) group [206 ± 9 vs. 210 ± 4 g, respectively, $P = $ not significant (NS)]. The weight gain during the 6-wk housing period was similar between groups (PAB 168 ± 9 vs. sham 172 ± 10 g, $P = $ NS). Right atrial and RV weight were increased in PAB rats, whereas left heart weights were unaffected (Table 1).
In many respects, the responses of the RV to dobutamine stimulation were similar in PAB and sham rats. End-systolic RV pressures increased somewhat in both groups, but only significantly in PAB rats at each level of dobutamine stimulation. $V_{es}$ decreased in response to dobu-2.5 in both groups, but the response was only significant in sham rats ($P < 0.001$, PAB: $P = 0.32$). At dobu-5, the $V_{es}$ increased nonsignificantly in PAB rats ($P = 0.18$) and was unaltered in sham rats. Further analysis did not reveal any statistical trends in the response of RV $V_{es}$ between PAB and sham rats at the two levels of dobutamine stimulation (all $P \geq 0.20$). RV $dP/dt$max increased at each level of dobutamine in both groups. RV SW, however, tended to decrease at dobu-2.5 and subsequently increased at dobu-5. All three PV relations increased significantly in response to dobu-2.5 in both groups (Fig. 2). In contrast, at dobu-5, all three PV relations demonstrated no further significant alterations compared with dobu-2.5. Although end-systolic elastance (Ees) decreased slightly in both groups ($P \geq 0.34$) and PRSW and $dP/dt$max-$V_{es}$ increased slightly ($P \geq 0.32$), no differences in the response between PAB and sham could be demonstrated ($P > 0.50$). Together, these results indicate an increased RV contractile response at dobu-2.5, without a further increase at dobu-5.

The LV of the PAB animals also responded in a similar fashion to dobutamine stimulation compared with its sham counterparts. LV end-diastolic and end-systolic pressures did not change significantly as a result of dobutamine stimulation. The LV $V_{es}$ and end-systolic volumes decreased in both groups equally, mainly as a result of the first dosage of dobutamine. Furthermore, the LV $dP/dt$max increased in both groups, whereas the stroke work remained unaltered. LV contractility increased in both sham and PAB rats, as represented by an increase in the slopes of the PV relations (Fig. 2). At dobu-5, only the LV of sham animals was capable of increasing contractility, as represented by an increase in $E_{es}$ and $dP/dt$max-$V_{es}$. In summary, the LV contractile response to dobutamine stimulation was similar for both groups at dobu-2.5, and the response of the PAB animals at dobu-5 was blunted.

**DISCUSSION**

Our findings indicate that 6 wk of RV pressure overload in our rat model resulted in enhanced baseline RV contractility and that RV contractile reserve was maintained, indicating a state of compensated RV hypertrophy. Furthermore, baseline LV contractility was unaffected, whereas the LV response to dobutamine stimulation was blunted. These data are in line with the observation that RV pressure overload did not result in alterations in systemic hemodynamic parameters and that overt signs of heart failure were absent.

Assessment of cardiac contractile state by load-independent parameters of LV function by means of the conductance catheter technique is well established (15, 29). It has been shown that the technique is applicable in the LV of large animals and humans, as well as small animals such as rats (7, 27). More recently, it was shown that, despite the complex geometry of the RV, this approach is also useful in characterizing RV contractile state and reserve in various conditions (3, 4, 8, 10, 17, 19, 25, 31). Some investigators have used this technique simultaneously in both LV and RV studying LV and RV responses and their interaction in various derangements of the normal circulation. The application of this biventricular conductance catheter technique so far has been limited to larger animals such as lambs (19). To our knowledge, this study is the first to apply the biventricular conductance catheter (multisegment) technique in small experimental animals such as rats.
In our study RV systolic pressure was increased to \( \sim 60\% \) of systolic LV pressure, a level of RV pressure overload that is commonly encountered in patients with residual abnormalities after palliative or corrective surgery for complex congenital heart disease. This resulted in a robust hypertrophic response (increasing RV mass 2-fold in the PA-banded rats; Table 1). We used three load-independent measures of contractility (ESPVR, PRSW, and dP/dt\(_{\text{max}}\)/V\(_{\text{ed}}\)) to determine the contractile state of both the RV and LV. These indexes all indicated a two- to threefold increase of RV contractility at baseline in the pressure-overloaded RV. Contractile reserve of the RV was demonstrated by a further increase of RV contractility at the lowest dose of dobutamine. Qualitatively, the responses of the RV were similar in the sham and PAB rats. Similarly, baseline LV contractility was the same in sham and PAB rats. At each level of dobutamine stimulation (2.5 and 5 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)), LV contractility increased in sham rats, but in PAB rats LV contractility did not increase further at the highest dose of dobutamine. Qualitatively, the responses of the LV were similar in both sham and PAB rats, except for a blunted response at the highest dose of dobutamine in PAB rats. RV volumes were slightly, but not significantly, lower in PAB rats, whereas LV V\(_{\text{ed}}\) tended to be somewhat lower in PAB rats (\( P = 0.09 \)).

The effects of pressure overload on RV contractile function have been studied previously under various conditions and by using various techniques. The onset of and the duration of the pressure overload determine the RV hypertrophic response and the effects on RV hemodynamics. For example, in patients with acute RV pressure overload, the RV dilates and stroke volume reduces. Initially, the increased end-diastolic RV volume serves to maintain cardiac output, but eventually RV dysfunction results in a diminished cardiac output (14, 26). In contrast, in young lambs with acute RV pressure overload, RV V\(_{\text{ed}}\) was unaltered, whereas RV contractile state increased (8, 9). These studies implicated RV homeometric autoregulation as an important immediate adaptation to RV pressure overload. In patients with chronic RV pressure overload, induced by either chronic obstructive pulmonary disease or primary pulmonary hypertension, magnetic resonance imaging studies have demonstrated an increased RV mass, a decrease of both RV and LV V\(_{\text{ed}}\), and a maintained RV systolic function (18, 32). Similar results have been obtained in experimental studies using the conductance catheter technique. Leeuwenburgh and coworkers (19) induced 8 wk of RV pressure overload in lambs by gradually increasing and maintaining RV pressures at systemic levels. In response, these lambs developed compensated RV hypertrophy as demonstrated by the increase of RV contractile indexes at a normal RV V\(_{\text{ed}}\). Furthermore, LV contractile function was not affected by the RV hypertrophy, since parameters of LV contractility were unaltered, whereas LV V\(_{\text{ed}}\) was decreased. The results from these previous studies are in line with the major findings from our study: in response to chronic RV pressure overload RV and LV contractile function is maintained at a normal or somewhat decreased RV V\(_{\text{ed}}\) and a somewhat decreased LV V\(_{\text{ed}}\).

In their study on chronic RV pressure overload, Leeuwenburgh and coworkers suggested that the increase in RV contractile state was out of proportion to the amount of hypertrophy, indicating a hypercontractile state of the RV. The results of our study are in line with the observations made by Leeuwenburgh and coworkers, although the increase in RV contractile state in our study seemed more in proportion with the amount of hypertrophy. When normalizing the three PV relations for RV weight, no difference was seen in two out of the three PV relations (Fig. 3). Although the PV relations increased significantly at dobu-2.5 in the sham animals, the qualitative response in sham and PAB animals to dobutamine was similar. This suggests that the intrinsic RV contractility of the PAB rats is similar or slightly higher compared with controls and that contractile reserve is maintained.

Notably, in the study in lambs, the LV contractile state was maintained at a lower LV V\(_{\text{ed}}\), suggesting a slight hypercontractile state of the LV as well. In our study, the LV V\(_{\text{ed}}\) also tended to be lower in the PAB rats. The finding that the baseline LV ESPVR was shifted leftward (LV volume intercept at 100 mmHg = 194 ± 22 vs. 114 ± 32 \( \mu \text{l} \), sham vs. PAB, \( P = 0.05 \)) also supports a hypercontractile LV function in the PAB rats. The drive for this hypercontractile state is unknown, but we found no signs of increased systemic sympathetic nervous stimulation. The LV response to dobut-2.5 stimulation was blunted in the PAB animals. Whether a down-regulation of LV \( \beta \)-adrenergic receptors plays a role in this response is uncertain. LV \( \beta \)-adrenergic receptors were down-regulated 4 wk after monocrotaline treatment in rats, whereas RV \( \beta \)-adrenergic receptors were unaffected (6). However, other studies have not found a downregulation in LV \( \beta \)-adrenergic receptors in RV hypertrophy (20, 21). Therefore, additional research to elucidate the mechanisms behind this LV blunted response is necessary.

![Graphs](http://ajpheart.physiology.org/)
Relevance of the Study

Many patients with corrected congenital heart disease, pulmonary hypertension, and/or chronic obstructive pulmonary disease have (residual) abnormal RV loading conditions, making them prone for the development of RV failure. One of the challenges is to identify the turning point from compensated RV hypertrophy to (irreversible) RV failure. In a previous study, we reported the changes in the myocardial protein expression in response to RV pressure overload (12). We demonstrated alterations in expression of metabolic proteins, compatible with a shift from fatty acid to carbohydrate metabolism. Furthermore, we found upregulation of several low-molecular-weight heat shock proteins and could demonstrate the phosphorylation of heat shock protein-27. The expression of several proteins seemed to be correlated with the degree of hypertrophy. For example, a phosphorylated heat shock protein-27 was positively correlated with the degree of hypertrophy ($r^2 = 0.89$, $P < 0.01$). This study demonstrates the feasibility of characterizing biventricular responses to RV pressure overload in a small animal model. This allows characterization of the progression of RV compensated hypertrophy to RV failure over time and correlation of these findings with alterations in myocardial protein expression. By using this approach, relevant changes in myocardial protein expression can be correlated with the onset of RV failure (11). Therefore, further longitudinal studies are needed to correlate hemodynamics with alterations in the myocardial proteome.

In conclusion, 6 wk of RV pressure overload in rats resulted in a state of compensated RV hypertrophy, whereas LV contractile state at baseline was not, or only marginally affected. Furthermore, this study demonstrated the feasibility of performing biventricular measurements using a multisegment conductance catheter technique in rats. This hemodynamic profiling of the preceding stages of heart failure in a small animal model will facilitate an integration between physiology and biochemistry, ultimately leading to a more accurate interpretation of heart hypertrophy and failure.

REFERENCES


