Distinct right ventricle remodeling in response to pressure overload in the rat

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Mendes-Ferreira P, Santos-Ribeiro D, Adão R, Maia-Rocha C, Mendes-Ferreira M, Sousa-Mendes C, Leite-Moreira AF, Brás-Silva C. Distinct right ventricle remodeling in response to pressure overload in the rat. Am J Physiol Heart Circ Physiol 311: H85–H95, 2016. First published May 6, 2016; doi:10.1152/ajpheart.00089.2016.— Pulmonary arterial hypertension (PAH), the most serious chronic disorder of the pulmonary circulation, is characterized by pulmonary vasoconstriction and remodeling, resulting in increased afterload on the right ventricle (RV). In fact, RV function is the main determinant of prognosis in PAH. The most frequently used experimental models of PAH include monocrotaline- and chronic hypoxia-induced PAH, which primarily affect the pulmonary circulation. Alternatively, pulmonary artery banding (PAB) can be performed to achieve RV overload without affecting the pulmonary vasculature, allowing researchers to determine the RV-specific effects of their drugs/interventions. In this work, using two different degrees of pulmonary artery constriction, we characterize, in full detail, PAB-induced adaptive and maladaptive remodeling of the RV at 3 wk after PAB surgery. Our results show that application of a mild constriction resulted in adaptive hypertrophy of the RV, with preserved systolic and diastolic function, while application of a severe constriction resulted in maladaptive hypertrophy, with chamber dilation and systolic and diastolic dysfunction up to the isolated cardiomyocyte level. By applying two different degrees of constriction, we describe, for the first time, a reliable and short-duration PAB model in which RV adaptation can be distinguished at 3 wk after surgery. We characterize, in full detail, structural and functional changes of the RV in its response to moderate and severe constriction, allowing researchers to better study RV physiology and transition to dysfunction and failure, as well as to determine the effects of new therapies.

pulmonary artery banding; pulmonary hypertension; right ventricle remodeling

NEW & NOTEWORTHY

Right ventricular (RV) adaptation to pressure overload differs depending on the degree of overload. In this work we present a thorough analysis of adaptive and maladaptive remodeling of the RV in response to pulmonary artery banding, allowing for future research to target a specific stage of RV remodeling.

PULMONARY ARTERIAL HYPERTENSION (PAH) is a progressive disease caused by exacerbated pulmonary vasoconstriction and vascular remodeling, resulting in increased pulmonary vascular resistances (PVR) and right ventricle (RV) dysfunction (59).

Current therapies attenuate disease progression, but long-term prognosis remains poor (54). Furthermore, new therapies continue to be focused on pulmonary artery (PA) vasodilation, leaving aside the RV function, which plays the most important role in the development of PAH (30), while side effects of some PAH-specific drugs might even include deterioration of RV function (66).

There is an increased need to develop RV-specific therapies and to understand RV adaptation to overload and progression to RV maladaptation and failure, although the RV’s complex anatomy, physiology, and deterioration in response to pressure overload (32) could represent a greater difficulty in achieving this goal.

The major cause of death in patients with PAH is RV failure (64). The prognostic value of RV structure and function has been gaining attention (17, 68), as larger RV volumes, low stroke volume (SV), reduced left ventricle (LV) volume, and RV ejection fraction (EF) are associated with increased morality. PAH patients treated with PAH-specific therapies present a poorer outcome when RV function has deteriorated, despite improved PVR (67), demonstrating the importance of evaluating and maintaining RV function in PAH.

New therapies for PAH should protect against RV maladaptation and failure (71), and experimental models in which those therapies can be tested are in huge demand. In recent years, a vast amount of data on how the RV adapts to PAH and pressure overload (1, 5, 9, 11), as well as some insight into the mechanisms underlying these changes (7, 25, 27), has emerged. Several PAH models, mainly the monocrotaline (MCT) and chronic hypoxia models, have been extensively used to study RV dysfunction and gain mechanistic insight into how the RV adapts (29) and have served as the basis for experimental drug development in PAH.

Using pulmonary artery banding (PAB), which entails application of a constriction to the pulmonary trunk, one can induce RV overload and RV hypertrophy (RVH) while preserving the pulmonary vasculature (37). This allows researchers to study RV-specific effects of pharmacological interventions independently from their effects on the pulmonary circulation (2, 8, 52, 57, 58, 63).

Several very interesting and comprehensive studies have adapted and characterized this procedure (20, 23, 35), first described as a model for compensated RVH in young rats (37), to larger-animal models (1) and different loading conditions (11).

Despite these advances, there is contradictory evidence regarding the RV response to the same constriction or the presence or absence of RV dysfunction and the extent to which this dysfunction occurs, limiting the choice of models to test RV-specific therapies.

In this work, using the PAB model, we applied two levels of pressure overload to understand how the RV responds to moderate and severe constriction.

Furthermore, using echocardiography, biventricular hemodynamics, in vitro evaluation of cardiomyocyte performance,
and histology, we fully characterize structural and functional changes of the RV in response to pressure overload. With this study we were able to demonstrate a stage of compensated/adaptive hypertrophy, in which RV-increased afterload results in little or no change in RV function, and a stage of decompensated/maladaptive hypertrophy, in which the RV develops systolic and diastolic dysfunction, with changes up to the cardiomyocyte level. This model will allow researchers to determine, over a short period of time (3 wk), the effects of pharmacological interventions in the two stages of RVH and adaptation to pressure overload, facilitating the development of new therapies focused on maintaining an adaptive RV.

MATERIALS AND METHODS

Our procedures followed the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1985), were certified by the Portuguese Veterinary Governmental Agency, and were approved by the Portuguese Foundation for Science and Technology (PTDC/SAU-FCF/100442/2008 and PTDC/DTD-PTO/0130/2012) and the faculty ethical committee (0420/000/000/2010). All animal handling was performed by trained researchers, who attended a Laboratory Animal Sciences course for certification according to the Federation of European Laboratory Animal Science Association.

PAB. Seven-week-old (180–200 g body wt) male Wistar rats (Charles River Laboratories) were anesthetized with 8% sevoflurane (SevoFlo, Abbott) inhalation. After intubation, animals were connected to a rodent ventilator (MouseVent G500, Kent Scientific), with tidal volume and respiratory rate adjusted to animal weight, and a Sevoflurane inhalation. After intubation, animals were conscious/awake, and the animal was allowed to recover on a heating pad. Suture integrity was checked for the next 3–4 days to ensure proper wound closure. Sham animals underwent the same protocol, except the suture around the PA was loosely tied. No mortality was observed 3 wk after nPAB and sPAB.

MCT-induced PAH. MCT-induced PAH was performed as previously described (45). Briefly, 7-wk-old male Wistar rats (180–200 g body wt) were injected with MCT (60 mg/kg sc; Sigma-Aldrich) and, after 3 wk, developed RVH and dysfunction.

Echocardiographic measurements. At 21 days after PAB surgery, all rats were anesthetized and intubated as described above and placed in the left lateral decubitus position, and the skin was shaved and depilated. An ultrasound system (Acuson Sequoia 512, Siemens) equipped with a 15-MHz probe (model 515L8) was used for the analysis. Temperature, heart rate (HR), pulse oximetry, and end-tidal CO2 were measured as described above. The echocardiographic parameters include LV end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD), RV end-diastolic dimension (RVEDD), RV end-systolic dimension (RVESD), right atrial area, tricuspid annular plane systolic excursion (TAPSE), RV free wall thickness, tissue Doppler-derived peak systolic velocity (μs'), and RV myocardial performance index (RV MPI).

Hemodynamic measurements. After the echocardiographic analysis, the animal was repositioned to the right lateral decubitus position. The common femoral vein was catheterized for fluid administration, and a left thoracotomy was performed; the pericardium and pleura were carefully dissected, and the phrenic nerve was severed. A 3-0 surgical silk was passed around the inferior vena cava for transient occlusion during the protocol, and pressure-volume catheters were inserted through the apex of the RV and LV (models SPR-869 and SPR-847, respectively, Millar Instruments) and positioned along the long axis. A flow probe (model MA2.5PSB, 2.5 mm, Precision Systems) was implanted around the ascending aorta and connected to an ultrasonic transit time-volume flowmeter (model TS420, transit-time perivascular flowmeter, Transonic Systems). The experimental preparation was allowed to stabilize for 15 min; blood loss during the procedure was replaced with a saline bolus. Pressure and volume signals were continuously acquired (model MVPS 300, Millar instruments), digitally recorded at a sampling rate of 1,000 Hz (model ML880 PowerLab 16/30, ADInstruments), and analyzed off-line (LabChart 7 Pro (ADInstruments) and PVAN 3.5 (Millar Instruments)).

Parallel conductance for the volume catheter was computed after an average of three bolus injections of 50 μL of hypertonic saline (10% sodium chloride). Calibration for factor alpha (field inhomogeneity) was determined through the cardiac output (CO) measured by the aortic flow probe and the ultrasonic transit time-volume flowmeter. At the end of the protocol, heparinized blood was collected for volume calibration in standard cuvettes (model P/N 910-1048, Millar Instruments).

Baseline hemodynamic parameters were HR, CO, RV and LV end-diastolic volume (EDV), end-diastolic pressure (ESP), end-diastolic pressure (EDP), SV, EF, arterial elastance (Ea), and the logistic time constant of isovolumic relaxation (τ). PVR was derived from ESP and CO, and cardiac index (CI) was derived from CO and body surface area. ESP- and EDP-volume slopes [end-systolic and end-diastolic elastance (Ees and Ead), respectively] were also determined. Ventricularcoupling was determined by dividing Ees by Ead. The mass-to-volume (M/V) ratio was determined by dividing RV mass by EDV. Wall stress (WS) (34) and pulmonary capacitance (1) were calculated as previously described.

Statometric and histologic analysis. After euthanasia, heart weight was measured, the RV free wall and LV + interventricular septum were carefully dissected and weighed separately. The tibia was collected and measured for tissue weight normalization. RV samples were fixed in 10% formaldehyde and embedded in paraffin. Hematoxylin-eosin- and Sirius Red-stained sections of the RV were used to measure cardiomyocyte cross-sectional area (CSA) and interstitial fibrosis content, respectively.

Isolated skinned cardiomyocytes. RV samples were defrosted in relaxing solution, mechanically disrupted, and permeabilized. Under microscopic view (model IX51, Olympus) and through imaging software (VSL 900B, Aurora Scientific), a single cardiomyocyte was attached to a force transducer (model 403A, Aurora Scientific) and a length controller (model 315C-I, Aurora Scientific). Cell length was digitally adjusted through custom-designed software (series 600A digital controller, Aurora Scientific), and steady-state passive force was measured at increasing sarcomere lengths (1.8–2.3 μm). Total force development was measured at a sarcomere length of 2.2 μm by varying submaximal free Ca2+ concentrations ([Ca2+]free) and active force was determined by subtracting passive force from total force. Force measurements were normalized to each cardiomyocyte CSA.

Statistical analysis. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software). One-way ANOVA with Tukey’s multiple-comparisons test was used for most of the data, with the exception of LVEI, RVEDA, and RVEDD, which were subjected to a Kruskal-Wallis test with Dunn’s multiple-comparison test, and the [Ca2+]free-sarcomere length curves, which were subjected to two-way
ANOVA with repeated measures with a Holm-Sidak multiple-comparisons test. Linear regression was used to graphically represent the Frank-Starling curves. Pearson’s correlation was used to determine the degree of association between fibrosis and RV EDP, where data distribution was normal. One phase-decay nonlinear relationship was used to graphically represent the capacitance-PVR curve.

RESULTS

RV structural changes after PAB. Rats subjected to PAB surgery developed RVH. Only animals subjected to sPAB showed a small, but statistically significant, decrease in body weight (Fig. 1A), a sign of a decompensated stage of RVH. RVH was inversely proportional to the size of the constriction, once the RV weight normalized to tibia length and Fulton index (RV/LV + S; Fig. 1, B and C) was increased in animals subjected to mPAB and further increased in animals subjected to sPAB. Morphometrically measured RVH was confirmed by increased cardiomyocyte CSA (Fig. 1, D and F), which was also increased in the mPAB group and to a greater extent in the sPAB group. However, only the sPAB group showed increased interstitial fibrosis (Fig. 1, E and F).

Pressure overload resulted in right heart dilation after 3 wk (Fig. 2J), with a mild nonsignificant increase in RV size in the mPAB group but a significant increase in the sPAB group, as quantified by a higher RVEDD (Fig. 2A), RVEDA (Fig. 2B), right atrial area (Fig. 2C), and LVEI (Fig. 2D). As a response to increased load, animals from the mPAB group developed a thicker RV free wall (Fig. 2E), which regressed in animals from the sPAB group, suggestive of decompensated RVH.

Pressure overload-induced RV and LV dysfunction. Animals from the sPAB group showed decreased RV function measured by echocardiography and by right heart catheterization. We observed RV failure only in animals from the sPAB group, where TAPSE (Fig. 2F), s' (Fig. 2I), and HR (377 ± 8 and 410 ± 14 beats/min for sPAB and Sham, respectively) were significantly decreased and RVMPI was higher (Fig. 2G), indicating systolic dysfunction in this group of animals. RV outflow tract excursion, although slightly decreased in the sPAB group compared with the Sham group, did not achieve statistical significance.

Right heart catheterization showed again that only the sPAB group developed signs of RV failure (Fig. 3, J–L). HR was...
decreased in this group of animals (Fig. 3A), and ESP was nearly threefold higher than in the Sham group, while mPAB animals presented a nearly twofold increase in ESP (Fig. 3B). RV filling pressures were increased only in the sPAB group (EDP; Fig. 3C), and RV dilation, as previously measured in the echocardiographic analysis, was observed only in the sPAB group. D: LV eccentricity index (LVEI) was increased in sPAB animals as a result of septal ablation and LV atrophy. E: RV free wall diastolic thickness (RVFWDT) was increased in mPAB animals and regressed to Sham levels in the sPAB group. F–I: RV function was decreased in sPAB animals, as measured by a decrease in tricuspid annular plane systolic excursion (TAPSE), an increase in RV myocardial performance index (RVMPI), a tendency toward RV outflow tract excursion (RVOTE) decrease, and a decrease in tissue Doppler-derived peak systolic velocity (′s′). Values are means ± SE of 3–8 rats per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Sham. #P < 0.05, ##P < 0.01 vs. mPAB. J: representative echocardiographic images of a short-axis view and an apical 4-chamber view of experimental groups. White dashed lines delineate RV, LV, and RAA.

Similar to the above-described observations, RVH resulted in systolic and diastolic dysfunction. Similar to the above-described observations, RVH resulted in systolic and diastolic dysfunction. Additionally, diastolic dysfunction was also present in this group, as measured by τ (Fig. 3H) and Eėd (Fig. 3I).

Similar to the RV, LV function was compromised only in the sPAB group. sPAB resulted in lower ESP (Fig. 4A) and higher filling pressure (EDP; Fig. 4B) in the LV of these animals, demonstrating interventricular dependence. LV unloading was also observed as LV filling was decreased, with a lower EDV (Fig. 4C) together with diastolic dysfunction, with a higher τ (Fig. 4D) in this group of animals.

sPAB results in cardiomyocyte dysfunction. Similar to the above-described observations, RVH resulted in systolic and diastolic dysfunction.
Diastolic dysfunction at the cardiomyocyte level, demonstrating that the effects of constricting the PA are not only structural changes (cardiomyocyte enlargement and fibrosis), but also intrinsic changes. Cardiomyocytes isolated from mPAB animals (Fig. 5A) showed increased active tension development compared with Sham animals (Fig. 5B), while sPAB cardiomyocyte active tension decreased compared with mPAB animals, revealing a depressed systolic function of the cardiomyocytes in this group. This was further supported by an increase in the EC50 for Ca2+/H11001 in the sPAB group, revealing decreased Ca2+/H11001 sensitivity (Fig. 5C), with a rightward and downward shift of the normalized tension-Ca2+/H11001 curve (Fig. 5D). Total tension was significantly higher in mPAB- than Sham- and sPAB-derived cardiomyocytes (Fig. 5F). Further- more, passive tension was significantly increased in the mPAB group and further increased in the sPAB group, demonstrating intrinsic cardiomyocyte stiffness, contributing to the diastolic dysfunction observed in these animals (Fig. 5F).

DISCUSSION

In this work we used two similar models of PAB, which resulted in an adaptive and a maladaptive RVH in response to pressure overload. Our results show that, at 3 wk after surgery, mild pulmonary constriction results in RV afterload increase, with compensated hypertrophy and preserved function and without compromise of LV function. On the other hand, at 3 wk after severe pulmonary constriction, RV afterload increase resulted in further development of RVH, with both systolic and diastolic dysfunction, and significant ventricular interaction, with compromise of LV filling. RV dysfunction is closely related to patient mortality, once hemodynamic and imagiological indexes of RV structure and function predict outcome in patients with PAH (72).

Pressure overload initiates improved contractile performance by increasing myocardial mass through cell hyperplasia (increased cardiomyocyte CSA) (40). As we have reported, cardiomyocyte size was increased in mPAB and to a greater
extent in sPAB. This resulted in an increase in contractility, as observed by higher Ees in the sPAB group, similar to that previously described (23). Animals from the mPAB group showed a trend toward increased Ees.

Despite an increase in Ees, PAB leads to systolic impairment (2, 11, 13), which was observed only in our sPAB group, in which both echocardiographic-derived (TAPSE, RVMPI, and s') and PV loop-derived (EF and CO) indexes of systolic function were decreased. Furthermore, a downward shift of the Frank-Starling curve [Fig. 6A; for the same preload (EDV), ejection was decreased (SV)] was observed in the sPAB group, showing systolic dysfunction. The increase in Ees in the sPAB group is associated with RV maladaptive remodeling, with increased chamber stiffness, which does not necessarily represent increased contractility (14). Furthermore, the volume intercept for the ESP-end-systolic volume relationship is increased, demonstrating that, for a given volume, sPAB animals develop decreased pressure (15).

RV adaptation to pressure overload is characterized by increased angiogenesis, hypertrophy, and contractility to preserve systolic function, and its transition to failure is underlined by capillary rarefaction (53, 62), dilation, increased stiffness, systolic impairment (56), and metabolic dysfunction (26–28, 62). Disruption of adaptive RV remodeling with decreased capillarization, increased inflammation, and fibrosis leads to RV maladaptive remodeling (56).

Decreased RV function in the sPAB group (Fig. 6B) might be a result of increased WS. Higher pressures, RV dilation, and RV free wall thinning result in increased WS, as previously reported in a rabbit model of RVH secondary to PAB (43), leading to cardiomyocyte contractile dysfunction, which is underlined by sarcomeric protein alterations (7).

Cardiomyocytes from the sPAB group presented decreased active force development and Ca²⁺/H¹ sensitivity, representative of the intrinsic cardiomyocyte dysfunction. In fact, cardiomyocytes isolated from the RV and LV behave differently in pressure overload (51). RVH with RV failure results in decreased maximal tension and decreased Ca²⁺/H¹ sensitivity (higher EC₅₀) compared with compensated RVH (24). Several groups have shown in other small-animal models (rabbit and ferret) that RV pressure overload results in impaired systolic function through a compromised force-frequency relationship (31, 69), which is associated with altered Ca²⁺ handling and sensitivity, and can be exacerbated by volatile anesthesia (33). This has also been confirmed in larger-animal models, where PAB severity was associated with Ca²⁺-handling disturbances and cardiomyocyte dysfunction (49), as observed in our experiments.

LV unloading was present in the sPAB group, with decreased LV volumes, increased LVEI, and septal ablation contributing to the decrease in CO observed in this group, as previously observed in the rabbit and pig model of RV pressure overload (39), demonstrating the importance of ventricular interdependence (3, 16, 36).

RVH is associated with decreased diastolic function (41), and recent studies have detailed the extent to which RV.
diastolic function is affected in PAB (10, 11, 22) and in human PAH (55, 60, 65). mPAB resulted in a slight increase in \( \tau \) and \( \frac{dP}{dt_{\text{min}}} \), while sPAB aggravated these changes and also resulted in increased EDP and \( E_{\text{ed}} \).

Interestingly, as previously described (11), active myocardial relaxation is completed before the end of diastole (Fig. 6C); therefore, diastolic dysfunction occurred through increased stiffness, and not through incomplete relaxation. This is confirmed by the upward shift of the length-passive tension curve, the increase in fibrosis, which is associated with PAB (6), and the positive correlation between EDP and fibrosis (12) (Fig. 6D).

Indeed, increased RV cardiomyocyte stiffness has been observed in samples from patients with PAH (55). Changes in cytoskeletal proteins, such as titin hypophosphorylation, might account for this increase in stiffness (55). In our work, cardiomyocyte stiffness was impaired in both PAB groups, with further aggravation in the sPAB group, similar to that previously shown in rabbits (43, 69).

Although LV \( E_{\text{ed}} \) did not change, both EDP and \( \tau \) were increased in the LV of sPAB animals, demonstrating diastolic dysfunction secondary to RV pressure overload in this group, similar to that previously described in the pig (16).

Adaptive remodeling is characterized by concentric hypertrophy, where the M/V ratio is increased and both systolic and diastolic function are preserved, while maladaptive remodeling is associated with eccentric hypertrophy and decreased systolic and diastolic function (72). This adaptation was described in human patients with PAH (5), in which the M/V ratio was used to distinguish patients with RV compensated hypertrophy and decompensated hypertrophy.

In our set of animals, the M/V ratio did not follow the pattern described previously, as we found an increase in the M/V ratio in the sPAB group (Fig. 6E) suggestive of an exaggerated hypertrophy without severe dilation. This might be associated with the degree of RV dysfunction, which is not as severe as in MCT-induced RV failure, and, thus, despite similar WS (Fig. 6B), the M/V ratio in the MCT group is decreased compared with sPAB animals (Fig. 6E). Interestingly, Trip et al. (65) showed that the M/V ratio was not decreased in patients with PAH and was actually increased in PAH patients with higher diastolic stiffness, similar to our sPAB group.

Using Yorkshire pigs subjected to pulmonary venous drainage banding, Aguero et al. (1) showed two distinct patterns of RV adaptation: compensatory hypertrophy, with preserved function, and decompensated hypertrophy with RV failure, with severe increases in PVR. Similar to this study, we observed a curvilinear relationship between PA capacitance and PVR (Fig. 6F), where a decrease in PA capacitance without an increase in PVR characterized adaptive hypertrophy (the mPAB group), while a steep increase in PVR was observed in maladaptive hypertrophy (the sPAB group). PA capacitance is associated with RV function (61) and serves as a strong predictor of RV failure (21), as its decrease is associated with higher mortality in heart failure (19).

The interesting finding that patients with PAH show a decrease in RV-PA coupling only when subjected to exercise testing (60) might explain why, even though most of the parameters in the sPAB group point toward maladaptive remodeling and RV failure, the RV-PA coupling is still not affected (Fig. 6G). Determining its adaptation to increased...
stress, such as stress echocardiography (42), might shed some light on this fact. Application of a 0.5- and a 0.6-mm constriction to the pulmonary trunk increased $E_{cs}$ in the compensated and decompensated stages, as previously observed in rodents (11) and larger-animal models (1, 3) of RV pressure overload and in humans with PAH (55, 60, 65). At the same time, PAB did not significantly change the $E_{cs}$-$E_{sa}$ ratio, although function was compromised (decreased EF) in these animals (2), suggesting a different adaptation of the RV to pressure overload in animals compared with human patients.

It has been shown (8, 9, 23) that the RV develops adaptive hypertrophy without heart failure only in response to pressure overload. To a certain point, our results corroborate those reported by Bogaard et al. (9). If we compare our mild constriction model with the MCT-induced PAH model, RVH occurs (52, 58). Although this is a limitation of our work, PAB animals at 6 wk after PAB surgery, and no other time point was studied.

Nonetheless, our data show that if the pressure overload is high enough, as in our severe constriction model, RV dysfunction will occur (Fig. 7).

Nevertheless, the decrease in function was not as severe in our sPAB group as in MCT-induced PAH, as the sPAB group shows a higher EF (Fig. 7D) and CO (Fig. 7E) than the MCT group, while pressure is higher (Fig. 7B) and RV afterload is similar (Fig. 7F). This observation demonstrates that a comprehensive hemodynamic evaluation is necessary to determine the full scope of RV function in this context, whereas measurement of RV pressures is not sufficient to fully characterize RV function and PA remodeling and dysfunction play a role in RV function, besides increasing afterload. The differences in RV function between the present study and previous reports using the same constriction (18-gauge needle) (8, 9, 23) might result from different surgical techniques (tightening of the suture around the needle might differ between laboratories), the time point at which functional evaluation was performed, and the strain, weight, and age of the animals. In fact, besides the obvious different adaptation to the same constriction (younger/smaller animals are subjected to a lesser degree of immediate constriction than older/larger animals), aging increases maladaptive remodeling of the PA (70). Furthermore, other studies in which a looser constriction (16-gauge needle) was used show that RV systolic dysfunction occurs (52, 58).

Additionally, our terminal evaluation was performed 3 wk after PAB surgery, and no other time point was studied. Although this is a limitation of our work, PAB animals at 6 wk after surgery showed the same function as PAB animals at 22 wk after surgery (9), which might lead us to conclude that RV (mal)adaptation occurs early and is stably maintained.

Hirata et al. (35) showed that use of a titanium clip to constrict the PA resulted in worsened RV function compared with ligation, despite using only TAPSE to quantify RV functional deficits.
One of the advantages of our approach is that, with PAB, the degree of constriction is adapted to the growth of the animal, which results in a gradual increase in RV pressure overload, consistent with the clinical evolution in patients with PAH. The two different models presented in this study distinguish adaptive remodeling, with systolic function preserved, from maladaptive remodeling, with systolic and diastolic dysfunction, in a predictable, constriction-dependent way.

Comparable to our study, application of PAB in medium-sized animals (rabbits, ferrets, and cats) has shown that the RV adapts to pressure overload, with hypertrophy and fibrosis (50), compromised RV function with impaired systolic (48, 50) and diastolic (38) function, and cardiomyocyte dysfunction (31, 33, 43, 69), resulting in LV unloading (39).

Larger-animal models of PAB have also been used to describe RV adaptation to pressure overload, allowing for use of innovative techniques, such as the adjustable PAB, which allows for the development of RVH and its reversal after constriction release (44, 46, 47), providing new insight into how the RV adapts after the constriction is removed and which pathways are associated with reverse remodeling. Additionally, by using a larger-animal model, other techniques, such as strain analysis, are more easily accessible (71). Dogs subjected to acute PAB and PAB release presented venriculoarterial coupling, together with myocardial apoptosis and inflammation (18). Severe PAB in dogs resulted in hypertrophy and right atrial and RV dysfunction (71), decreased HR, and decreased cardiomyocyte function (altered Ca²⁺ handling) (49), corroborating our sPAB group findings.

In sheep, severe PAB resulted in decreased RV systolic function and dilation and RV deformation compromise (as measured by 3-dimensional echocardiographic speckle tracking), decreased HR, and LV compromise (4). These findings in larger-animal models point to the differential adaptation of the RV to distinct pressure overload conditions, resulting in adaptive and maladaptive hypertrophy, as suggested by our data.

In this work we fully describe and characterize two models of PAB-induced RV pressure overload that developed adaptive or maladaptive remodeling 3 wk after PAB surgery. Our data show that a mild constriction results in hypertrophy with preserved function, while a severe constriction results in RV dysfunction with aggravated remodeling, namely, increased hypertrophy and fibrosis. The main novelty of our work is full characterization of RV function by use of echocardiography, pressure-volume technology, and in vitro isolated cardiomyocyte analysis to thoroughly describe the RV changes in response to pressure overload. This points to the utility of these models, in which, in just 3 wk, RV adaptation and maladaptation can be distinguished, allowing for a more comprehensive analysis of new drug targets in different stages of RV overload and failure vs. nonfailure.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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