Chronic hypoxia induces nonreversible right ventricle dysfunction and dysplasia in rats

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Bonnet, Pierre, Sébastien Bonnet, Julien Boissière, Jean-Loïc Le Net, Mathieu Gautier, Eric Dumas de la Roque, and Véronique Eder. Chronic hypoxia induces nonreversible right ventricle dysfunction and dysplasia in rats. Am J Physiol Heart Circ Physiol 287: H1023–H1028, 2004; 10.1152/ajpheart.00802.2003.—The purpose of this study was to evaluate the reversibility of right ventricular (RV) remodelling after pulmonary artery hypertension (PAHT) secondary to 3 wk of hypobaric hypoxia. A group of 10 adult male Wistar rats were studied and were the following: control normoxic (C), after 3 wk of normoxic recovery (N-RE). Mean pulmonary artery pressure was 11 ± 2 mmHg in the C group, 35 ± 2 mmHg in the CH group, and 14 ± 3 mmHg in the N-RE group. RV function was assessed by echocardiography. In the CH group, the pulmonary flow measured in Doppler mode depicted a midsystolic notch and a decrease of the pulmonary acceleration time compared with control [17 ± 1 vs. 34 ± 1 ms (n = 10), respectively; P < 0.05]. RV thickening measured in M-mode was apparent in the CH group compared with the control group (2.84 ± 0.40 vs. 1.73 ± 0.26 mm (n = 10), P < 0.05). In the N-RE group, the RV wall was significantly thinner compared with the CH group [1.56 ± 0.08 vs. 1.73 ± 0.26 mm (n = 10), P < 0.05]. The calculated RV diameter shortness fraction was not different between the CH group and C group (34 ± 4.2% vs. 36 ± 2.8%) but decreased in the N-RE group [20 ± 2.4% (n = 10), P < 0.01]. The E-to-A wave ratio on the tricuspid Doppler inflow was significantly lower in the CH group and N-RE group compared with the C group [0.70 ± 0.8 and 0.72 ± 0.1 vs. 0.88 ± 0.2 (n = 10), respectively; P < 0.05]. In the isolated perfused heart using the Langendorff method, RV compliance was increased in the CH group and decreased in the N-RE group. In the N-RE group, fibrous bands with metaplasia were observed on histological sections of the RV free wall. We conclude that PAHT induces nonreversible RV dysfunction with dysplasia.

Using a chronic hypoxic (CH) rat model, we studied the evolution of RV changes in systolic and diastolic function using high-frequency transthoracic echocardiography (TTE). TTE is a useful technique for the evaluation of RV pathology and allows in vivo assessment of cardiac function in small animals such as the rat. We used this technique to assess RV function during recovery, following the return of chronically hypoxic rats to normoxic conditions. PAP was measured by right catheterization to quantify the severity of pulmonary hypertension. The use of the Langendorff method permitted an approach of diastolic changes in isolated and perfused hearts. Histological evaluation evaluated potential structural alterations.

METHODS

Animal Model

Adult male Wistar rats (n = 10) aged from 7 wk were exposed for 3 wk to chronic hypoxia (CH) in a hypobaric chamber (50 kPa), and the animals were then housed during 3 wk in a normoxic environment. Experiments were performed before, within 1 h of removal from the CH, and then after rats recovered under a normoxic environment (normoxic recovery, N-RE) for 3 wk. A duration of 3 wk for CH exposure for normoxia remission was chosen on the basis of previous experiments (2). A separate group of five rats of the same age was housed with the same care but were not exposed to CH to evaluate the spontaneous evolution of echographic parameters. These echocardiographic measurements were made at the same times (7, 10, and 13 wk).

In separate experiments (28 rats) both echocardiographic measurements and PAHT were assessed by measuring PAP by catheterism. To avoid repeated catheterism, experiments have been realized on separate series of 10 normoxic (C) rats (7 wk old), 9 CH rats (10 wk old), and 9 N-RE rats (13 wk old). Each animal had only one echocardiogram and one catheterization. In these experiments, after death of the animals, hearts were removed and cleaned of connective tissue, and the right and left ventricle were weighed separately.

Another group of adult male Wistar rats (n = 21) was used for isolated and perfused heart preparation to investigate diastolic function in normoxic (C; n = 7), hypoxic (HC; n = 7), and recovery (N-RE; n = 7) conditions. Histological examinations were performed on separate experiments of five rats, each submitted to the same exposure protocol (C, CH, N-RE).

These investigations were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996) and European Directives (86/609/CEE).

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Echocardiographic Technique

The rats were lightly sedated with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (7.5 mg/kg). Transthoracic twodimensional, M-mode and Doppler imaging were performed in the same 10 animals (CH and N-RE) and in the 5 rats of the control group. We used an Acuson C256 ultrasonographic system (Mountain View, CA) with a 12-MHz transducer. M-mode and Doppler tracings were recorded at a sweep speed of 200 mm/s. M-mode measurements were performed according to recommendations of the committee on M-mode standardization of the American Society of Echocardiography. All the studies were performed by an experienced sonographer who was blinded to the treatment group.

M-mode measurements. For accurate and reproducible measures, simultaneous recording of one-lead ECG was used to determine the moment of the diastole and systole. M-mode measurements of RV dimensions were performed in the parasternal short-axis view. RV free wall thickness in diastole was measured in a parasternal modified long-axis view. This view was chosen because it offered the most convenient image of the RV free wall in this rat model. The shortness fraction (SF) was calculated as SF = (RVDd − RVSD)/(100/RVDd) (where RVDD is diastolic diameter and RVSD is systolic diameter). RV surface was determined in the bidimensional mode in apical four-chamber view in telediastole and in telesystole. The four-chamber apical view allowed a reproducible image of the RV to be obtained and delineating its surface with a cursor around of the cavity (14). Left ventricular (LV) shortness fraction and septal thickness was also evaluated in parasternal long-axis view.

Doppler imaging. All evaluations of PA flow were performed with a (maximal) sweep speed of 200 mm/s. Tricuspid regurgitation (TR) was assessed in apical four-chamber view with color Doppler. Pulsed-wave Doppler of pulmonary outflow was recorded in the parasternal view at the pulmonary valve level. The pulmonary acceleration time (PAHT) was measured from the beginning of the pulmonary flow to its onset. The duration of the PA flow defined the ejection time. E and A waves represent the protodiastolic component (passive RV filling) and telediastolic component (atrial-dependent RV filling) of the tricuspid flow and the RV filling.

RV relaxation and filling was assessed on the Doppler tricuspid inflow by the ratio of peak E wave to peak A wave velocities and deceleration time of the E wave. All these parameters defined the diastolic function.

Catheterism

PAP was measured in closed-chest rats by means of a polyethylene catheter inserted into the right jugular vein and manipulated through the RV into the PA.

Isolated and Perfused Hearts

The Langendorff isolated and perfused heart preparation was used to investigate diastolic function. Rats were divided in three separate groups, first (control, n = 7), second (CH, n = 7), and third (N-RE, n = 7). The rats were then anesthetized with 40 mg/kg of pentobarbital sodium injected intraperitoneally and antiagugulated with 1,500 IU heparin sodium (obtained from Leo Laboratories; St. Quentin, France). The thorax was opened, and the heart was rapidly excised and immediately cooled in iced Krebs buffer. Hearts were perfused, at a constant flow of 3 ml/min, by an aortic cannula delivering warm (37°C) modified phosphate-free Krebs-Henseleit solution containing (in mM) 118 NaCl, 5.9 KCl, 1.75 CaCl₂, 1.2 MgSO₄, 0.5 EDTA, 25 NaHCO₃, and 16.7 glucose. The perfusate was gassed with 95% O₂-5% CO₂, which resulted in a PO₂ above 600 mmHg at the level of the aortic cannula and a buffer pH of 7.4. The PA was transsected to facilitate coronary venous drainage. RV pressure was monitored from a water-filled latex balloon placed through the right atria appendage and connected to a Statham P23 pressure transducer. The balloon was filled with water at different increased volumes with an electric syringe to obtain the pressure-volume relationship of the RV.

Histological Measurements

Histological examinations were performed in three groups of five rats each that were submitted to the same exposure protocol (CH, N-RE). After death, each animal’s heart was immediately dissected, and the right and left ventricles were placed in a formalin solution and cut into slices (40 μm, long axis) for examinations. Structural changes were qualitatively evaluated by an investigator blinded to the treated groups.

Statistical Analysis

All values are expressed as means ± SE. Intergroup differences were assessed by a repeated-measure ANOVA or factorial ANOVA, as appropriate. Post hoc analysis used Fisher’s test (NCSS 5.0). Pearson’s correlation coefficient was used to describe the relationship between mean PAP and PAAT and RV wall thickness. A value of P < 0.05 was considered statistically significant; a nonsignificant result is shown by NS.

RESULTS

Hypoxic PAHT Evaluation

Catheterization was performed successfully in all animals. After 3 wk in hypoxic conditions, rats developed a moderate PAHT. The mean PAP was measured to 35 ± 2 mmHg (n = 9) in the CH group and 11 ± 2 mmHg (n = 10) in the control group. The PAHT decreased to the normal after 3 wk of recovery to 14 ± 3 mmHg (n = 9) (Fig. 1), a value not significantly different from the control group.

Echocardiography did not allow direct estimation of PAHT because TR was present in only 2% of the cases. Therefore, we used indirect evaluation of PAHT by ejection flow analysis and RV thickness measurement. Measurements of PA flow were possible in all animals. Two features of PA flow were found in the CH group: 1) a change in the shape of the PA waveform, and 2) a decrease of the PAAT. As shown in Fig. 2, a PAA mid-systolic notch was prominent in the CH group, whereas it was absent in the control and N-RE groups. The PAAT values significantly decreased in the CH group compared with the control rats (17 ± 1 vs. 34 ± 1 ms, n = 10, P < 0.05). In the recovery group, PAAT returned to the normal (32 ± 2 vs. 34 ± 2 ms, P = 0.007).
1 ms, \( n = 10, P = \text{NS} \). In comparing the value of PAAT with the mean PAP value measured by catheterization in all groups, we noticed that the PAAT correlated linearly \((r = -0.71, n = 28, P < 0.0001)\) with mean PAP value (Fig. 3).

RV free wall thickening was a consistent echocardiographic feature of pulmonary hypertension in the CH group. Mean wall thickness in the CH group was significantly increased compared with the control value \((2.84 \pm 0.4 \text{ vs. } 1.73 \pm 0.26 \text{ mm, } n = 10, P < 0.05)\). In the N-RE group, the RV wall was significantly thinner than the RV wall in CH rats \((1.56 \pm 0.08 \text{ vs. } 2.84 \pm 0.4 \text{ cm, } n = 10, P = \text{NS})\). RV diameter shortness fraction used as a systolic function index was not significantly different between control and CH groups \((34 \pm 4.2\% \text{ vs. } 36 \pm 2.8\%, \text{ respectively; } n = 10, P = \text{NS})\) (Fig. 5). Recovery phases induced a significant decrease of the RV diameter shortness fraction in the N-RE groups compared with both control and CH groups \((20 \pm 2.4\% \text{ vs. } 34 \pm 4.2\% \text{ and } 36 \pm 2.8\%, n = 10, P < 0.05)\). RV diastolic function was assessed on tricuspid pulsed Doppler flow assessment. The tricuspid (E/A) ratio was significantly lower in both CH and N-RE groups than in the control group \((0.70 \pm 0.8 \text{ vs. } 0.72 \pm 0.1, n = 10, P < 0.05)\) (Fig. 6).

Effects of Chronic Hypoxia on RV Systolic and Diastolic Function

In diastole RV, the surface was not different between the control and CH groups \((0.24 \pm 0.02 \text{ vs. } 0.27 \pm 0.03 \text{ cm}^2, n = 10, \text{NS})\). In the N-RE group, the RV surface was slightly increased compared with control \((0.30 \pm 0.05 \text{ vs. } 0.24 \pm 0.02 \text{ cm}^2, n = 10, P = \text{NS})\). RV diameter shortness fraction used as a systolic function index was not significantly different between control and CH groups \((34 \pm 4.2\% \text{ vs. } 36 \pm 2.8\%, \text{ respectively; } n = 10, P = \text{NS})\) (Fig. 5). Recovery phases induced a significant decrease of the RV diameter shortness fraction in the N-RE groups compared with both control and CH groups \((20 \pm 2.4\% \text{ vs. } 34 \pm 4.2\% \text{ and } 36 \pm 2.8\%, n = 10, P < 0.05)\). RV diastolic function was assessed on tricuspid pulsed Doppler flow assessment. The tricuspid (E/A) ratio was significantly lower in both CH and N-RE groups than in the control group \((0.70 \pm 0.8 \text{ vs. } 0.72 \pm 0.1 \text{ vs. } 0.88 \pm 0.2, n = 10, P < 0.05)\) (Fig. 6).
Deceleration time of the E wave increased from 43 ± 9 ms in the control and to 65 ± 10 ms (n = 10, P < 0.05) in the CH and 68 ± 10 ms (n = 10, P < 0.05) in the N-RE groups. These results show a marked alteration in RV relaxation induced by CH, and this alteration was not reversible. LV function was studied in addition to RV. No LV function alterations were observed (LVSF = 27 ± 3% in CH group vs. 26 ± 2% in normal). CH or N-RE did not induce LV hypertrophy. Moreover, no significant modifications occurred in all cardiac parameters between the beginning of the exposure protocol (week 7) and the end of the protocol (week 13) in the separated groups of five rats of which none were exposed to CH and recovery.

RV compliance was assessed by the pressure-volume relationship. As shown in Fig. 7, RV compliance was significantly increased in the CH groups compared with control conditions, whereas in the N-RE group a significant decrease of compliance was observed.

**Effects of Chronic Hypoxia on RV Structure**

The ratio between the RV and LV weight was of 0.30 ± 0.03 in normoxic rats (n = 10) versus 0.60 ± 0.09 in the CH rats (n = 10) and 0.31 ± 0.03 in the N-RE rats (n = 10). Histological study of the heart clearly showed a profound remodelling of the RV structure. Macroscopically, we observed increased thickness of the RV wall in the CH group. In the N-RE group, we noticed that roughly 70% of the rats had abnormal thinness of the RV wall particularly located in the outflow track (Fig. 8), and the weight of the RV went back to the control value. Microscopic analysis first confirmed the thickening of the RV wall in the CH group and the slight dilatation of the RV in the N-RE group. In the CH groups, we noted a loss of myocardial cells within the free wall of the right ventricle and an early fibrosis with infiltration of a small amount of mononuclear inflammatory cells. Interestingly, in the N-RE group we observed a loss of myocardial tissue and the development of fibrous bands with areas of cartilaginous and osseous metaplasia compared with the control rats (Fig. 8).

**DISCUSSION**

**Echocardiography Evaluation of PAHT**

This study examined the feasibility of using high-frequency TTE to monitor the development of pulmonary hypertension in the chronic hypoxic rat model. Data showed that the echocardiography images in rats are of good quality and feasible when obtained with a modern ultrasonographic system. In a previous study, high-frequency echocardiography had been validated for cardiac rat evaluation with a reproducibility of the measure that was in the same range as in humans (1, 3, 12). In our study, PAHT was mild to moderate and tricuspid TR was not observed (13). A greater frequency of TR has been reported in a monocrotaline-induced PAHT rat model where the mean pulmonary pressure was greater (~65 mmHg) than that observed in the present study (11). Several indirect parameters such as changes in PA waveform, PAAT, and RV-free wall thickness are found to be consistent with the trend of developing pulmonary hypertension in rats (15). The changes in the PA waveform and the PAAT were found to be an early event in the development of pulmonary hypertension (11). In our model, PAAT is linearly correlated with the mean PAP showing that PAAT is a good way to evaluate PAHT in the chronically hypoxic rat. The main change in RV during PAHT is the increase of the free wall thickness. Our results show that the increase of RV wall thickness is linearly correlated to the mean...
PAP pressure. This measure is easy to obtain with a parasternal modified view. Doppler tissue imaging exploration was not implemented in our system. In human studies, this technique has provided interesting results to characterize RV function and could be used in the future for rat PAHT evaluation (5, 6, 9, 10).

RV Remodelling

Right systolic function evaluated by RV shortness was unchanged in the CH group, but diastolic function was impaired. Diastolic dysfunction was confirmed by the isolated heart study. Echographic findings confirmed these results by an E/A ratio decrease (16). Because the cardiac rate in the three groups was similar, the E/A ratio decrease could not be attributed to heart rate change. Because no alteration in LV function was observed, RV dysfunction is not due to global cardiac insufficiency. One major result of our study is that this dysfunction persists after 3 wk of recovery with slight hypertrophy and RV dilatation, despite hemodynamic normalization. Even more, the pathological alteration seems to be worsened by the normoxic recovery period.

Histological examination confirmed the persistence and worsening of histological alterations with the loss of myocardial tissue, metaplasia, and fibrosis. The pathological mechanisms of these persistent alterations remain to be explored. CH could have an impact because overload alters RV oxidative metabolism that might explain the RV dysfunction and the fibrosis infiltration that we have observed (4). In a previous study, RV overload was obtained by PA banding, and no inflammatory infiltration and no dysfunction was observed during the phase of hypertension. In our model intercellular infiltration is present in the CH group and worsened in the remission group. However, the absence of LV function alteration suggests that hypoxemia is not the only factor and that the pressure overload could also initiate the pathological process that alters the RV function. Hypertrophy in response to pulmonary hypertension leads to an increase of intermyocytes distance by cellular hypertrophy (18). The stretch of the wall by pressure overload or by intermyocytes distance augmentation could have an impact on the myocytes loss of the RV. This type of mechanism is an actual hypothesis for arrhythmogenic RV dysplasia pathogenesis. In arrhythmogenic RV dysplasia, the main abnormalities are a fatty tissue replacement that is not observed in our case, which limits the comparison (8). However, with regard to our results, we focus on the possible implication of pressure overload in persistent RV alteration. Obviously, we cannot speculate on the specific role of reoxygenation on the worsening of the RV lesions. It would be interesting to study the influence of the different times of hypoxia/remission protocol in a further study.

In conclusion, our study confirmed the value of noninvasive cardiac evaluation of cardiac function by high-frame echocardiography in rat. RV thickness measurement and pulmonary flow pattern modification as the diminution of PAAT are accurate indirect parameters for PAHT evaluation when tricuspid regurgitation is absent. After 3 wk in hypoxic condition and normoxic remission phase of PAHT, our study shows that systolic and diastolic dysfunction persist because of RV structural alterations.

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