Chronic hypoxia effects active tone differently in the pulmonary arteries of Brown-Norway, Sprague-Dawley, and Fawn-Hooded rats

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Introduction
Chronic alveolar hypoxia is a well established stimulus for invoking pulmonary arterial hypertension and vascular remodeling in the rat. One major component of the vascular remodeling is abnormal contraction of smooth muscle cells into small peripheral arteries (downstream) that under normal conditions are devoid of muscle. This component is represented in histological studies as the percent muscularization. A second component is the increase in thickness of the muscle mass in the normally muscular arteries (upstream). Histologically, it is quantified as percent wall thickness, normalized by the vessel’s external diameter. Both of these components of vascular remodeling will increase pulmonary vascular resistance (PVR) through intimal narrowing, further muscularization, and reduced vessel recruitment. Upon cessation of flow, the pressure maintained by the pulmonary vasculature reflects a critical closing pressure. In the alveolar region, an increase in the critical closing pressure indicates less compliant vessels in the alveolar region. To identify the muscle tone present in the pulmonary vascular tree at the end of the exposure period, we performed hemodynamic testing in isolated, perfused lungs and measured vascular resistance and critical closing pressure both before and after adding papaverine to the perfusate. Various rat strains express different susceptibilities to this hypoxic challenge. Fawn-Hooded Sprague-Dawley Brown-Norway strain, which is used as a model of idiopathic pulmonary hypertension, has been shown to be highly susceptible to chronic hypoxic exposure, leading to severe pulmonary hypertension.

To investigate if there are differences in baseline and remodeling tone levels that are strain dependent, we studied PVR and CCP in Fawn-Hooded (FH), Sprague-Dawley (SD) and Brown-Norway (BN) rats.

Methods
For each strain studied (FH, BN, and SD), rats were randomly housed for 21-days in an isobaric hypoxic exposure chamber (10% O2) or kept under similar but normoxic conditions (N:3 for each group). A chamber flow rate of at least 15 liters was maintained during the exposure period and the rats were housed in racks and drink ad libitum. Body weight and fluid consumption were monitored daily. On the 21st day the rats were anesthetized with sodium pentobarbital (50 mg/kg ip), heparinized (200 IU) by right ovarian injection and a blood sample taken for hematocrit determination. The trachea and pulmonary artery were cannulated, the heart excised and lungs removed and surrounding tissue trimmed. The heart was dissected to determine right ventricular free wall versus left ventricular plus septum weight determination. The lungs were suspended by the cannulae, inflated, and perfused by recirculating a physiological salt solution containing 7% bovine serum albumin plus glucose. Pressure transducers were placed to monitor main pulmonary artery and airway pressure. The pulmonary vein was open to the atmosphere (0 mmHg). The lungs were first perfused at 10 ml/min and ventilated at 60 breaths/minute with a 15% O2, 6% CO2 in N2 gas mixture, 3 mmHg end-expiratory pressure and 8 mmHg end-inspiratory pressure for several minutes. Ventilation was halted and the lung held at an airway pressure of 12 mmHg. The arterial pressure (P1) versus flow relationship was then measured at flow rates of 40, 30, 20, 15, 10, 5 ml/min. The pump was stopped and after approximately 20 seconds when a plateau was reached, the critical closing pressure was measured. Ventilation was resumed, 6 mg of papaverine hydrochloride was added to the reservoir (approximately 0.6 mg/kg), and the lung perfused at 7 ml/min for approximately 2 minutes. Ventilation was again halted and pulmonary arterial pressure versus flow measured a second time at the 6 flow rate mentioned above. Finally, the perfusate in the arteries was replaced with perfusate hormone for X-again continuous and angioscopic images performed on the lung. Statistical evaluations were performed with Sigma Stat (P<0.05). Hemodynamic comparisons were made using a two-way repeated measures ANOVA test.

Results

Water consumption

Body weight

Pulmonary vascular resistance

Right ventricular hypertrophy

Critical closing pressure

Summary/Conclusions

Planar angiograms of the lungs reveal vessel dropout and less arterial distension after chronic hypoxic exposure. Our group has also quantified these changes in CT data from isolated rat and pig lungs. Chronic hypoxic exposure also results in a significant increase in PVR and right ventricular hypertrophy in the lung on in situ studies. The FH rat displays the largest increase in PVR after chronic hypoxic exposure. After chronic hypoxia, PVR is significantly larger in the FH rat compared to either the SD rat or the BN rat. Treatment of chronic hypoxia exposed rat lungs with papaverine significantly decreased PVR in the three strains studied. The largest effect of papaverine on tone is seen in the SD strain and the smallest effect is seen in the BN strain. Rat strain appears to be the largest hypoxic tone loss than observed in chronic hypoxia. There was no significant difference in PVR of normoxic control lungs treated with papaverine; however, the change in shape of the PVR plot demonstrated a normotensive response, particularly for the FH and BN rats. Before and after treatments with papaverine, there was a larger decrease in the downstream resistance. This is also implied by the significant decrease in CCP after papaverine administration in the normotic rats. The SD normoxic rat has a significantly larger CCP than either the FH or BN rat. There was a significant increase in CCP caused by chronic hypoxic exposure in the FH rat. Although not significant, chronic hypoxic treated or control animals had a decreased CCP in the BN strain. The FH strain is the only strain studied that had a significant decrease in CCP after chronic hypoxic exposure and treatment with papaverine.

Discussion

It is interesting that although the FH rat strain undergoes the largest increase in PVR it does not experience the largest increase in right ventricular hypertrophy. This suggests that the vascular control may be different in vivo compared to isolated perfused lungs used in this study. In that other humoral factors are also involved. Chronic hypoxic exposure results in an increase in vascular tone. The tone is preserved differently in the FH rat strain studied. BECAUSE CCP is significantly different in these strains, it is likely that peripheral vascular remodeling, including muscle extension into downstream vessels, is also different between the strains and therefore the tone of this muscle displays strain-dependent.

Acknowledgements
Support by the Department of Veterans Affairs, NIH HL19298, and the W.M. Keck Foundation.