

This document only includes an excerpt of the corresponding thesis or dissertation. To request a digital scan of the full text, please contact the Ruth Lilly Medical Library's Interlibrary Loan Department (rlmlill@iu.edu).

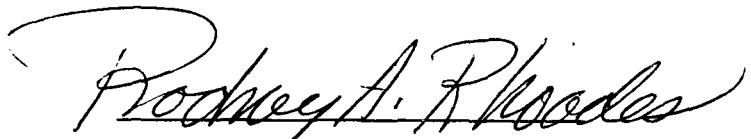
ALTERED PULMONARY ARTERIAL SMOOTH MUSCLE WITH
CHRONIC HYPOXIA-INDUCED PULMONARY HYPERTENSION

Janet E. Roepke M.D.

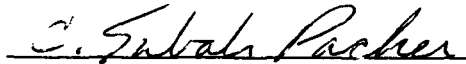
Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Department of Physiology and Biophysics
Indiana University

May 1993

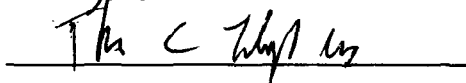
Accepted by the Graduate Faculty, Indiana University,
in partial fulfillment of the requirements of the degree of Doctor
of Philosophy.



Rodney A. Rhoades, Ph.D.
Professor and Chairman
Department of Physiology
and Biophysics



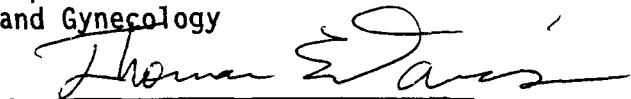
C. Subah Packer, Ph.D.
Professor
Department of Physiology
and Biophysics



Thomas C. Lloyd, M.D.
Professor
Department of Physiology
and Biophysics
Department of Medicine



Richard A. Meiss, Ph.D.
Professor
Department of Physiology
and Biophysics
Department of Obstetrics
and Gynecology



Thomas Davis, M.D., Ph.D.
Professor
Department of Pathology

DOCTORAL
COMMITTEE

January 1993
Date of oral examination

ABSTRACT

Pulmonary hypertension is a primary event leading to the development of right ventricular failure and respiratory failure. The principal stimulus which evokes pulmonary hypertension is chronic alveolar hypoxia. This results in remodeling of the vessel walls, including an increase in wall thickness by hypertrophy and hyperplasia of the pulmonary arterial smooth muscle (PASM) and an increase in the deposition of connective tissue. At present there is insufficient knowledge regarding the role that the vascular smooth muscle plays in pulmonary hypertension. The hypothesis of the proposed study is that hypoxia alters the biochemical, structural, and contractile properties of PASM. Such alterations may be the underlying mechanisms for the development and/or the maintenance of chronic pulmonary hypertension. Experiments were designed to determine whether or not hypoxia-induced pulmonary hypertension alters: 1) the maximum isometric tension, 2) optimum resting tension, 3) sensitivity and/or reactivity to agonists, 4) structural properties of the pulmonary arterial smooth muscle, and 5) relative amounts of contractile proteins in the pulmonary arterial smooth muscle. To induce pulmonary hypertension, young adult male rats were made hypoxic by experimentally lowering the percent inspired oxygen. Pulmonary arterial segments were isolated from the rat lungs and then assessed for changes in reactivity to agonists by measuring isometric tension development in response to various agonists and

comparing the dose-response curves of the control and hypertensive groups. Gel electrophoresis was used to assess changes in contractile protein isoforms. Histology was used to correlate the structural changes with changes observed in the pharmacology and biochemistry studies. This study provides evidence of decreased isometric tension development relative to the amount of muscle in pulmonary arteries from rats with pulmonary hypertension. This along with the generalized decrease in reactivity to agonists and a trend toward an increase in the non-muscle myosin isoforms, suggests that the mechanism by which hypoxia induced pulmonary hypertension is maintained is likely due to a shift in muscle phenotype to a synthetic, less-contractile phenotype.

TABLE OF CONTENTS

LIST OF FIGURES.....	xii
LIST OF TABLES.....	xiv
LIST OF ABBREVIATIONS.....	xv

CHAPTER I

INTRODUCTION

A. General Introduction.....	1
B. Pulmonary Hypertension Clinical.....	7
C. Mechanical Properties of Arterial Smooth Muscle.....	16
D. Pharmacological Properties of Pulmonary Arterial Smooth Muscle.....	19
E. Arterial Wall Structure in Pulmonary and Systemic Arteries and Changes with Hypertension.....	32
F. Biochemistry of Smooth Muscle.....	39

CHAPTER II

RATIONALE AND OBJECTIVES.....	42
-------------------------------	----

CHAPTER III

MATERIALS

A. Animals.....	44
B. Chemicals.....	44
C. Materials.....	47
D. Equipment.....	48

CHAPTER IV

METHODS

A. Experimental Protocol for Hypoxic Exposure.....	51
B. Demonstration of Presence of Pulmonary Hypertension	
1. Measurement of Hematocrit.....	53
2. Measurement of Right Ventricular Pressure.....	53
3. Measurement of Right Heart Hypertrophy.....	54
C. Rat Pulmonary Arterial Ring Preparation	
1. Isolation of Arterial Ring Segments.....	54
2. Endothelium Removal.....	57
D. Force Development Studies	
1. Measurement of Force Development.....	60
2. Calculation of Arterial Cross-Sectional Area...60	
3. Determination of Optimal Resting Tension.....	64
E. Pharmacological Studies	
1. Determination of Dose-Response Curves.....	65
a. Norepinephrine.....	67
b. Angiotensin II.....	69
c. Serotonin.....	69
d. Adenosine.....	70
e. Potassium Chloride.....	71
2. Assessment of Agonist Sensitivity and Reactivity.....	72
F. Microscopic and Morphological Studies	

1. Preparation of Tissue.....	72
2. Micrographs.....	75
3. Morphometric Analysis.....	76
G. Electrophoresis Studies	
1. Extraction of Myofibrillar Protein from PASM...78	
2. Gel Electrophoresis.....	79
3. Determination of Myofibrillar Protein Content..82	
H. Statistical Analysis.....	84

CHAPTER V

RESULTS

A. Verification of Presence of Pulmonary Hypertension	
1. Body Weight.....	85
2. Hematocrit.....	85
3. Right Ventricular Pressure.....	85
4. Right Heart Hypertrophy.....	88
B. Force Development Studies	
1. Optimal Resting Tension.....	91
2. Maximal Isometric Tension Development.....	99
C. Pharmacological Studies	
1. Norepinephrine.....	105
2. Angiotensin II.....	112
3. Serotonin.....	117
4. Adenosine.....	123
5. Potassium Chloride.....	131
D. Electrophoresis Studies	
1. Characterization of Myofibrillar Proteins of Control Pulmonary Arterial Smooth Muscle...133	
2. Changes in Myofibrillar Proteins with Chronic Hypoxia.....135	
E. Microscopic and Morphometric Studies	
1. Qualitative Observations.....	138
2. Quantitative Observations.....	146

CHAPTER VI

DISCUSSION.....	150
-----------------	-----

CHAPTER VII

SUMMARY.....	206
--------------	-----

BIBLIOGRAPHY.....	210
-------------------	-----

CURRICULUM VITAE

ABSTRACT.....	172
---------------	-----