

F

AD-A283 190

3E

Form Approved  
OMB No. 0704-0188

Public reporting burden  
gathering and maintain  
collection of information  
Davis Highway, Suite 120

onse, including the time for reviewing instructions, searching existing data sources,  
mation. Send comments regarding this burden estimate or any other aspect of this  
arters Services, Directorate for Information Operations and Reports, 1215 Jefferson  
get, Paperwork Reduction Project (0704-0188), Washington, DC 20503.



1. AGENCY USE (

3. REPORT TYPE AND DATES COVERED

4. TITLE AND SUBTITLE  
CARBON MONOXIDE Does Not Modulate Pulmonary  
VASCULAR Reactivity in Isolated Rat Lungs

5. FUNDING NUMBERS



6. AUTHOR(S)

James M. Cantrell

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Colorado State University

8. PERFORMING ORGANIZATION  
REPORT NUMBER

94-108

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

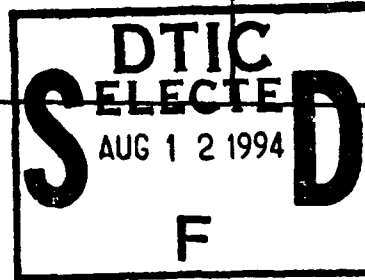
DEPARTMENT OF THE AIR FORCE  
AFIT/CI  
2950 P STREET  
WRIGHT-PATTERSON AFB OH 45433-7765

10. SPONSORING / MONITORING  
AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT  
Approved for Public Release IAW 190-1  
Distribution Unlimited  
MICHEAL M. BRICKER, SMSgt, USAF  
Chief Administration

12b. DISTRIBUTION CODE



13. ABSTRACT (Maximum 200 words)

14. SUBJECT TERMS

15. NUMBER OF PAGES

46

16. PRICE CODE

17. SECURITY CLASSIFICATION  
OF REPORT

18. SECURITY CLASSIFICATION  
OF THIS PAGE

19. SECURITY CLASSIFICATION  
OF ABSTRACT

20. LIMITATION OF ABSTRACT

## GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet *optical scanning requirements*.

### Block 1. Agency Use Only (Leave blank).

**Block 2. Report Date.** Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

**Block 3. Type of Report and Dates Covered.** State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

**Block 4. Title and Subtitle.** A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

**Block 5. Funding Numbers.** To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

**Block 6. Author(s).** Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

**Block 7. Performing Organization Name(s) and Address(es).** Self-explanatory.

**Block 8. Performing Organization Report Number.** Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

**Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es).** Self-explanatory.

**Block 10. Sponsoring/Monitoring Agency Report Number.** (If known)

**Block 11. Supplementary Notes.** Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

**Block 12a. Distribution/Availability Statement.** Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

### Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

**Block 13. Abstract.** Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

**Block 14. Subject Terms.** Keywords or phrases identifying major subjects in the report.

**Block 15. Number of Pages.** Enter the total number of pages.

**Block 16. Price Code.** Enter appropriate price code (*NTIS only*).

**Blocks 17. - 19. Security Classifications.** Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

**Block 20. Limitation of Abstract.** This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

**THESIS**  
**CARBON MONOXIDE DOES NOT MODULATE PULMONARY VASCULAR**  
**REACTIVITY IN ISOLATED RAT LUNGS**

Submitted by

James M. Cantrell

Department of Physiology

Accession For		
NTIS CRA&I	<input checked="" type="checkbox"/>	
DTIC TAB	<input type="checkbox"/>	
Unannounced	<input type="checkbox"/>	
Justification		
By		
Distribution /		
Availability Codes		
Dist	Avail and/or Special	
A-1		

In partial fulfillment of the requirements  
for the Degree of Master of Science  
Colorado State University  
Fort Collins, Colorado  
Summer, 1994

**94-25342**



*6298*


**94 0 1 1 09 7**

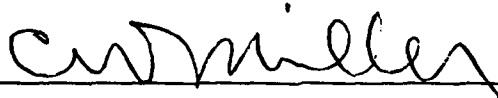
COLORADO STATE UNIVERSITY


July 7, 1994

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY JAMES M. CANTRELL ENTITLED **CARBON MONOXIDE DOES NOT MODULATE PULMONARY VASCULAR REACTIVITY IN ISOLATED RAT LUNGS** BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

  
\_\_\_\_\_

  
\_\_\_\_\_

  
\_\_\_\_\_

Advisor

  
\_\_\_\_\_

Department Head

## ABSTRACT

### CARBON MONOXIDE DOES NOT MODULATE PULMONARY VASCULAR REACTIVITY IN ISOLATED RAT LUNGS

Recent studies have demonstrated that the gas, nitric oxide (NO), when inhaled in low concentrations acts as a vasodilator in the pulmonary vasculature. Due to the physical and chemical similarities between NO and carbon monoxide (CO), we speculated that acute, low concentration exposure to CO would have similar effects in the isolated rat lung. Therefore, the purpose of this study was to determine the role of CO (200 and 1,000 ppm) in modulating hypoxia- and angiotensin II-induced pulmonary vasoconstriction, using isolated salt-perfused (Earle's Salt Solution; + 4 g% Ficoll) lungs of male Sprague Dawley rats (CON). Pulmonary hypertensive rats (ALT), induced by simulated altitude exposure (15,000 ft; 4,572 m; 430 mmHg for 32-48 days), were also used to determine the effects of CO in a remodeled pulmonary vascular bed. Two protocols were used in this study. Protocol A consisted of an initial hypoxic (6% CO<sub>2</sub>, balance N<sub>2</sub>, for 10 min) and AII (0.4µg) challenge, followed by

a carbon monoxide + hypoxia (6% CO<sub>2</sub>, 945 ppm CO, balance N<sub>2</sub>) and AII challenge. A final hypoxic and AII challenge concluded this protocol. All lungs in this protocol received 0.4µg AII. Protocol B consisted of two initial hypoxic (6% CO<sub>2</sub>, balance N<sub>2</sub>) challenges, followed by a third hypoxic challenge which included an AII injection at min 5 of the hypoxic exposure. Next, a CO + hypoxic challenge was given with an AII injection at min 5 of exposure. A final fourth hypoxic challenge with AII injection concluded this protocol. In this protocol, AII injections were also given between hypoxic challenges. Three CON rat lungs and all ALT rat lungs received 0.2µg AII. The remaining five CON rat lungs received 0.4µg AII injections.

Right ventricular hypertrophy (RV/BW ratios: ALT = 0.76 ± 0.02; CON = 0.52 ± 0.02), (RV/(LV+S) ratios: ALT = 0.41 ± 0.01; CON = 0.27 ± 0.01), and polycythemia (hematocrit: ALT = 55.8 ± 1.2; CON = 45.9 ± 0.8) were evident in the ALT rats. These data suggest that simulated altitude exposure induced pulmonary hypertension and consequent pulmonary vascular remodeling.

In protocol A, CO did not significantly affect pulmonary vascular responsiveness to acute hypoxia in either CON or ALT rats. There were also no significant differences in pulmonary pressor responses to AII injections in CON or ALT lungs immediately following the acute CO + hypoxic exposures.

Similarly, in protocol B, CO did not significantly affect pulmonary pressor responses to acute hypoxia in CON or ALT rats. In CON, but not ALT rats, responses to AII given during hypoxic challenges ( $1.7 \pm 0.4$ ) were significantly ( $P < 0.05$ ) smaller than comparable AII injections given between hypoxic challenges ( $4.0 \pm 0.6$ ). However, in both CON and ALT rats, CO had no significant affect on responses to AII injections which were given during or between hypoxic challenges. Therefore, acute CO exposure (at concentrations of 200 and 1000 ppm) does not appear to modulate pulmonary vascular reactivity to hypoxia in CON and ALT rat lungs. These data also suggest that acute CO exposure does not attenuate pulmonary pressor responses to AII administered either during or between hypoxic challenges.

James M. Cantrell  
Department of Physiology  
Colorado State University  
Fort Collins, Colorado  
Summer, 1994

**TABLE OF CONTENTS**

<u>Chapter</u>		<u>Page</u>
	ABSTRACT.....	iii
I	INTRODUCTION.....	1
II	LITERATURE REVIEW.....	4
III	RATIONALE AND SPECIFIC AIMS.....	8
IV	MATERIALS AND METHODS.....	10
V	RESULTS.....	17
VI	DISCUSSION.....	39
VII	SUMMARY.....	47
	CONCLUSIONS.....	48
	RECOMMENDATIONS.....	48
	REFERENCES.....	50

**CHAPTER I**  
**INTRODUCTION**

The pulmonary circulation is unique in that it offers very little resistance to blood flow, with pulmonary vascular resistance (PVR) being only one-tenth of that of the systemic circulation (1). Another interesting difference between the two circulations is that, while the vessels of the systemic circulation generally vasodilate in response to reduced oxygen tension (2), the pulmonary circulation responds to reduced alveolar oxygen tension by local active vasoconstriction of the arterioles in very close proximity to the alveoli (3). This local increase in vascular resistance, termed hypoxic pulmonary vasoconstriction (HPV), serves to divert blood flow from poorly ventilated areas of the lung to better ventilated areas of the lung, thus improving ventilation to perfusion matching and gas exchange (2). However, in situations where the whole lung is chronically exposed to hypoxia, such as chronic obstructive lung disease, cystic fibrosis and high altitude exposure, HPV causes pulmonary hypertension and may result in right ventricular hypertrophy (4,5).

HPV has been extensively studied since it was first demonstrated in 1946 by von Euler and Liljestrand (6). However, the underlying mechanisms of HPV remain unknown (7). Humoral mediators, such as histamine, eicosanoids, and catecholamines, have been suggested as possible mediators of HPV (2). Other researchers suggest that an indirect mechanism, which causes suppression of endothelium-derived relaxing factors (EDRFs) or prostacyclin, may result in HPV (8). Another hypothesis is that the pulmonary vascular tone is not regulated by oxygen, per se, but by the change in oxygen concentration or the cell energy state (7). In addition, experiments in isolated animal and human pulmonary arteries suggest that HPV is mediated by a direct effect of hypoxia on pulmonary vascular smooth muscle (8).

The recent focus on endogenous mediators of HPV has revealed that a gas, nitric oxide (NO), now considered to be EDRF (9), might play an important role in regulating pulmonary vascular smooth muscle tone in the normoxic and hypoxic lung (10). Another very similar gas, carbon monoxide (CO), may also play a role in modulation of pulmonary vascular smooth muscle tone. Ramos et al. (11) demonstrated that CO relaxes rat coronary and aortic vascular smooth muscle by an endothelium-independent mechanism. Carbon monoxide has also been shown to alter smooth muscle contraction by modulating intracellular  $Ca^{++}$  concentrations in rat aortas (12). In a study of chronic exposure of neonatal

rats to CO or high altitude by Penney et al. (4), those rats exposed to CO did not develop pulmonary hypertension as did the altitude-exposed rats, suggesting that CO may have a vasodilatory effect on the pulmonary vessels. However, the data are limited on the specific effects of low doses of inhaled CO on pulmonary vascular reactivity.

The present study was designed to investigate the pulmonary vascular effects of acute CO exposure in concentrations of 200 and 1000 ppm, with particular interest in the actions on HPV. The information gained from this research should contribute to the data available on the specific effects of low-dose inhaled CO on the pulmonary vasculature and provide further insight into the mechanisms of HPV. The significance of this area of research is emphasized by the prevalence and severity of chronic hypoxic diseases such as, chronic obstructive lung disease, cystic fibrosis and primary pulmonary hypertension, which can affect persons of any age.

## CHAPTER II

### LITERATURE REVIEW

Historically, carbon monoxide (CO) has been considered to be a highly toxic gas and instances of CO poisoning have been found in early Greek and Roman literature (13). The first understanding of the pathophysiology of CO originated with the work of Claude Bernard in 1857 when he attributed the toxic effects of CO to tissue hypoxia (13). In 1895, Haldane described the underlying mechanisms of CO toxicity when he demonstrated that CO reversibly interacts with hemoglobin in the blood to form carboxyhemoglobin, blocking the binding of oxygen with hemoglobin, and resulting in tissue hypoxia (14).

CO is a low molecular weight, colorless, odorless, tasteless gas that is slightly lighter than air (13,15). It is produced environmentally by the incomplete combustion of carbon-containing compounds and is a major constituent of motor vehicle exhaust and tobacco smoke (13). CO is also formed endogenously under physiological conditions (16) from NADPH-dependent enzymatic lipid peroxidation and from NADPH-dependent oxidative heme destruction, which is catalyzed by heme oxygenase (17).

The cleavage of heme by heme oxygenase results in the production of biliverdin and CO (17,18).

CO activates endogenous soluble guanylyl cyclase resulting in an increased production of guanosine 3', 5' cyclic monophosphate (cGMP) (16). In addition, cGMP-dependent protein phosphorylation is found in many tissues, including smooth muscle, and regulation of the level of cGMP may result in either relaxation or contraction of smooth muscle, with both events mediated by protein phosphorylation (16,19). Previous studies have demonstrated that relaxation of aortic smooth muscle is accompanied by an increase in intracellular cGMP (11). Similarly, Furchgott and Jothianandan (20) demonstrated that CO produces increases in cGMP levels in the rabbit aorta and dog coronary artery. An elevation in cyclic nucleotide levels has been associated with a decrease in cellular calcium content and relaxation of vascular smooth muscle (19). In tissues other than vascular smooth muscle, cGMP can regulate ion channels via both direct and indirect mechanisms (19). All of the above mentioned effects of CO appear to be independent of the tissue hypoxic actions of CO caused by binding to hemoglobin (16). Although this evidence suggests the possibility that CO has a physiological role in control of vascular smooth muscle, the data on the effects of CO specific to pulmonary vascular smooth muscle are limited. Duke and Killick (21) in 1952 reported vasodilation of pulmonary blood vessels in isolated, perfused cat lungs

exposed to CO. More recently, Tucker and Penney (22) reported an increase in pulmonary vascular reactivity in rats chronically exposed to simulated high altitude (3505m), but not in rats chronically exposed to 500 ppm CO.

Due to the biochemical and physiological similarities of NO and CO, the present study was modeled after a recent study investigating the dose-response relationship of NO in modulating pulmonary pressor responses to hypoxia and angiotensin II (AII) in isolated salt-perfused rat lungs. In the study by Rich et al. (23), the effects of acute inhaled NO exposure on hypoxic pulmonary vasoconstriction were determined by exposing the isolated lungs of male Sprague-Dawley rats to initial hypoxic challenges (100% N<sub>2</sub>) followed by NO (20, 50, 100, or 1000 ppm) plus hypoxic challenges which were in turn followed by another hypoxic challenge. This sequence was then repeated. The study also investigated the effects of NO on pulmonary pressor responses to angiotensin II by injecting AII after a sequence of hypoxic and NO plus hypoxic challenges as described above. The results of the study demonstrated that NO significantly attenuated the peak pulmonary pressor response to hypoxia (measured by pulmonary artery pressure change) in isolated rat lungs. In addition, NO significantly attenuated AII-induced vasoconstriction in isolated rat lungs.

Therefore, in order to determine the effects of acute inhaled CO on the pulmonary pressor responses to hypoxia and AII, the present study used similar protocols.

Thus, considering the proposed new role for CO as an endogenous modulator of systemic vascular smooth muscle tone and the lack of current data on the effects of CO on the pulmonary vasculature, the present study provided a unique opportunity to study the effects of acute CO exposure on the pulmonary vascular responses to vasoconstrictor challenges.

## CHAPTER III

### RATIONALE AND SPECIFIC AIMS

#### Rationale

Previous studies have shown that HPV is distinct and reproducible, despite some degree of variability in all species, and that humans and rats exhibit very similar responses to hypoxia. In addition, CO has been demonstrated to relax vascular smooth muscle in various species. Therefore, using an isolated perfused rat lung model is appropriate for investigating the effects of low dose carbon monoxide as a possible modulator of pulmonary vascular tone.

#### Statement of Hypothesis

Inhaled CO in concentrations of 200 and 1000 ppm will modulate hypoxic pulmonary vascular reactivity as evidenced by reduced vasoconstrictor responses to hypoxia and angiotensin II.

#### Specific Aims

The specific aims of the present study were to determine in isolated perfused rat lung, the effects of CO (200 and 1,000 ppm) on:

1. the magnitude and time-course of hypoxic pulmonary vasoconstriction;

2. the subsequent pulmonary vasoconstriction induced by angiotensin II;
3. angiotensin II-induced vasoconstriction during a hypoxic challenge; and
4. hypoxia-and angiotensin II-induced vasoconstriction in lungs with remodeled vascular beds caused by long-term high altitude exposure.

**CHAPTER IV**  
**MATERIALS AND METHODS**

**Animals**

Forty-two male Sprague-Dawley rats (Harlan, Inc.), weighing 380-470g, were used for the experimental procedures. The animals were housed at the Painter Center for Laboratory Animals on the Colorado State University campus in clear plastic cages (Thoren) in climate-controlled rooms (25°C, 12-hr light-dark cycle). The rats received Purina rodent chow and water ad libitum.

After a 7-day acclimatization period to the altitude of Fort Collins, CO (4,984 ft; 1,520 m;  $P_B = 635$  mm Hg), 16 rats were removed from the Painter Center and exposed to simulated altitude (15,000 ft; 4,572 m;  $P_B = 430$  mm Hg), in a 16 ft<sup>2</sup> hypobaric chamber for 4 weeks. The animals were housed 2 to a cage and up to 8 cages were placed in the chamber. The chamber was recompressed every other day to clean the cages and replenish food and water. Each of the altitude (ALT) animals was removed from simulated altitude 18-24 hrs prior to experimentation.

The remaining animals (control, CON) remained in the Painter Center until the day of the experiment.

#### **Isolated Lung Preparation**

On the day of the experiment, each rat was anesthetized with sodium pentobarbital (60mg/100g b.w., i.p.). The trachea was isolated and cannulated, and the rat was ventilated at 65 breaths/min with a Harvard rodent respirator (model 681). Inspiratory pressure was set at 8.5 cm H<sub>2</sub>O and expiratory pressure at 3 cm H<sub>2</sub>O. The chest was opened by median sternotomy. Heparin (100 µl) was administered by intracardiac injection, and 0.5 ml of right ventricular blood was extracted for measurement of hematocrit. A small incision was made in the right ventricle and the pulmonary artery was cannulated with a small-bore (17 gauge) steel cannula. Perfusion of Earle's salt solution (with 4g% Ficoll and 2g%/100 ml NaHCO<sub>3</sub>), utilizing a peristaltic pump (Gilson, Minipuls 2), was initiated at a low flow rate. The left ventricle was cannulated with a wide-bore (12 gauge) steel cannula to provide effluent perfusate flow to a heated (37°C) reservoir. The lungs were isolated and suspended in a heated (37°C) water-jacketed chamber. Perfusion was set at a constant rate of 3.5 ml/min/100g body weight. Pulmonary artery pressure (Ppa) was monitored and recorded (Gilson, model ICT-2H recorder) by a transducer (Statham, model 23-1D) connected to the small-bore cannula positioned in the

pulmonary artery. The isolated perfused lung was allowed to equilibrate under normoxic (21% O<sub>2</sub>, 6% CO<sub>2</sub>) conditions for 15 min prior to beginning the experimental protocol.

**Protocol A (Table 1)**

To determine the effects of CO on HPV, 8 control rats (CON) and 6 altitude-exposed rats (ALT) were studied using the following protocol. A 15 min normoxic equilibration period preceded a 5 min hypoxic (6% CO<sub>2</sub>, balance N<sub>2</sub>) exposure (HX-1), a 5 min recovery period, and an intra-arterial injection of AII (0.4μg). This sequence was repeated (HX-2, REC, and AII). After this sequence, the lung was exposed for 10 min to a hypoxic gas mixture which contained carbon monoxide (CO+HX; 6.08% CO<sub>2</sub>; 945 ppm CO, balance N<sub>2</sub>, certified, General Air Service and Supply, Fort Collins, CO). This exposure was likewise followed by a 5 min recovery period and an AII injection. The protocol ended with another sequence of hypoxia (HX-3), recovery, and AII injection (Table 1). Each animal served as its own control.

**Protocol B (Table 1)**

To determine the effects of CO on hypoxia- and AII-induced vasoconstriction, 8 CON rats and 6 ALT rats were studied using the following protocol. A 15 min normoxic equilibration period was followed by a 5 min hypoxic exposure (HX-1, 6% CO<sub>2</sub>, balance N<sub>2</sub>), a 5 min recovery period, and an intra-arterial injection of AII. This sequence was repeated once

more (HX-2). Next, the lung was exposed to a 10 min hypoxic exposure (HX-3) during which an intra-arterial injection of AII was given at minute 5 of the exposure. This challenge was also followed by a 5 min recovery period and an AII challenge during normoxia. This sequence was repeated by substituting the CO+HX (945 ppm CO) gas for the hypoxic gas. The protocol ended with a repeat of the hypoxic sequence without CO in the gas mixture. Each animal served as its own control.

### Pulmonary Pressor Rise and Decline Rates During Hypoxia

Initial pressure rise (from baseline) and decline (from peak) rates (mm/sec) were assessed in three sequential challenges (HX-2, CO+HX, and HX-3) in protocol A to determine the effect of CO on the time-course of hypoxic pulmonary vasoconstriction, with particular interest on the "responsiveness" of the pulmonary blood vessels.

### Perfusate pH, PO<sub>2</sub>, and PCO<sub>2</sub>

Perfusate samples were obtained during the last 2 min of normoxic, hypoxic and CO+HX gas exposures. The partial pressures of oxygen (PO<sub>2</sub>) and carbon dioxide (PCO<sub>2</sub>) and the pH of the perfusate were measured using an acid-base analyzer (Radiometer ABL-30).

### Heart weights and Hematocrit

At the termination of each lung perfusion, hearts were dissected and divided into right ventricle (RV) and left ventricle plus septum (LV+S) to determine the degree of pulmonary hypertension induced by altitude exposure. The segments were then weighed to determine the ratios of right ventricle to body weights (RV/BW) and right ventricle to left ventricle plus septal weights (RV/(LV+S)). Blood samples obtained during the lung isolation procedure were used to determine hematocrit via microcentrifugation (Damon IEC MB).

Statistical Evaluation

A one-way factorial analysis of variance was used to determine differences within hypoxic/CO+hypoxic and AII challenges. Significance was determined by Fisher's Protected Least Significant Difference (PLSD) test. The alpha level was set at 0.05. Hematocrit,  $RV/(LV+S)$ , and  $RV/BW$  were compared by paired  $t$ -tests. Data are expressed as mean  $\pm$  SE.

Table 1. Protocols for isolated perfused rat lung model.

Protocol A													
	EQ	HX-1	REC	AII	HX-2	REC	AII	CO+HX	REC	AII	HX-3	REC	AII
TIME (min)	15'	5'	3'	5'	10'	3'	5'	10'	3'	5'	10'	3'	5'
GROUP													
CON				0.4			0.4			0.4			0.4
ALT				0.4			0.4			0.4			0.4

Protocol B																
	EQ	HX-1	REC	AII	HX-2	REC	AII	HX-3	REC	AII	CO+HX	REC	AII	HX-4	REC	AII
TIME (min)	15'	5'	5'	5'	5'	5'	5'	10'	5'	5'	10'	5'	5'	10'	5'	5'
GROUP																
CON (n=5)				0.4			0.4	0.4		0.4	0.4		0.4	0.4		0.4
CON (n=3)				0.2			0.2	0.2		0.2	0.2		0.2	0.2		0.2
ALT				0.2			0.2	0.2		0.2	0.2		0.2	0.2		0.2

"EQ" denotes normoxic equilibrium period. "HX" denotes hypoxic pressor challenge. "REC" denotes normoxic recovery period. "CO+HX" denotes carbon monoxide and hypoxic pressor challenge. "AII" denotes angiotensin II ( $\mu$ g) pressor challenge. "HX+AII" denotes hypoxic pressor challenge with AII injection at min 5 of the challenge. "CO+HX+AII" denotes carbon monoxide and hypoxic pressor challenge with AII injection at min 5 of the challenge.

## CHAPTER V

### RESULTS

#### RESPONSE TO HIGH ALTITUDE EXPOSURE

Previous studies have demonstrated that prolonged simulated high altitude exposure results in polycythemia, evident by increased hemoglobin and hematocrit levels (24). There is also a remodeling of the pulmonary vasculature, with medial thickening and increased wall to lumen ratios (25). Consequently, right ventricular work is increased, resulting in right ventricular hypertrophy and concomitant pulmonary hypertension (26). In order to determine whether or not the altitude-exposed rats in this study did indeed develop the above-mentioned responses, the following data were obtained.

##### **Heart Weights** (Table 2)

The ratio of right ventricle to left ventricle plus septal (RV/(LV+S)) weights, and the ratio of right ventricle to body weights (RV/BW) were significantly greater ( $P < 0.05$ ) in ALT rats compared to CON rats.

##### **Hematocrit** (Table 2)

Hematocrits (ratio of red blood cell volume to volume of whole blood) were significantly greater ( $P < 0.05$ ) in ALT rats compared to CON rats.

### PULMONARY VASCULAR PRESSOR RESPONSE

In isolated perfused rat lungs exposed to hypoxia, the typical response is for the pulmonary artery pressure (Ppa) to rise (indicative of an increase in vascular tone) and remain elevated as long as the hypoxic stimulus is applied. However, if the hypoxic stimulus is longer than several minutes, there is very often a decrease in vascular tone with a concomitant reduction in Ppa (roll-off) (Figure 1). In contrast, angiotensin II injections in isolated rat lungs cause a rapid rise in Ppa to a peak value followed by an equally rapid fall to resting tone (Figure 1). In protocols A and B, two and three alternating hypoxia and AII challenge sequences, respectively, were administered to the isolated lungs to establish baseline pressor responses prior to the CO+HX or CO+HX+AII challenges.

#### **Responses to Acute Hypoxia/CO+Hypoxia-Protocol A**

Preliminary experiments using 200 ppm CO showed no measurable effect on pulmonary pressor responses to acute hypoxia or AII. Therefore, the focus of this study was narrowed to the use of 1000 ppm CO.

#### Control Rats (Figure 2)

In CON isolated rat lungs, the responses to the first hypoxic challenge (HX-1) were significantly less ( $P < 0.05$ ) than HX-2, HX-3, and CO+HX. There was no significant

difference between the peak pressor response to CO+HX ( $6.1 \pm 0.8$ ) (mean  $\pm$  SE) compared to HX-2 ( $6.9 \pm 0.8$ ) or HX-3 ( $4.6 \pm 0.9$ ).

Altitude Rats (Figure 3)

In ALT lungs, there were no significant differences among ALT lungs pressor responses to HX-2, ( $10.4 \pm 2.3$ ) and/or CO+HX ( $10.8 \pm 1.8$ ), or HX-3 ( $8.9 \pm 2.4$ ).

**Responses to Angiotensin II (0.4  $\mu$ g) Protocol A**

Control Rats (Figure 4)

In CON lungs injected with AII (0.4 $\mu$ g) between exposures to hypoxia, or CO and hypoxia, a general non-significant decrease in the pressor responses to AII was observed after the peak response of AII-2. Responses to the fourth AII injection were significantly ( $P < 0.05$ ) less than responses to the second AII injection.

Altitude Rats (Figure 5)

In ALT lungs injected with AII (0.4 $\mu$ g) as described above, there were no significant differences among the responses to AII injections after exposure to hypoxia compared to responses after exposure to hypoxia and CO. ALT lungs exhibited a progressive decline in responsiveness to repeated injections of AII (0.4 $\mu$ g).

**Hypoxic Pulmonary Vasorelaxation Protocol A**

In the isolated perfused rat lung, the increase in Ppa caused by hypoxia is followed by a reduction in vascular tone during continued exposure to hypoxia (hypoxic pulmonary

vasorelaxation). The following results describe the effects of CO (945 ppm) on the time-course of hypoxic pulmonary vasoconstriction in isolated rat lungs.

Hypoxic pulmonary vasorelaxation was assessed as the decline in Ppa from peak Ppa to the end of hypoxia during hypoxic and CO plus hypoxic gas exposures in the isolated lungs. The declines in Ppa (roll-offs) were not significantly different among HX-2, CO+HX, and HX-3 in CON rats (Figure 6). Similar to CON rats, there were no significant differences in roll-offs of HX-2, CO+HX, and HX-3 in ALT rats (Figure 7).

#### **Effects of CO on The Time-Course of HPV**

In order to determine the effect of acute CO exposure on the rate (mm/sec) of rise (from baseline) and the rate of decline (from peak) in pulmonary pressor responses to HPV, measurements of the recorded pulmonary artery pressure changes were made on three sequential challenges (HX-2, CO+HX, and HX-3) of CON and ALT lungs in protocol A. There were no significant differences in the rate of rise or rate of decline of pulmonary artery pressures during hypoxia, without or with CO, in CON or ALT lungs (Table 3). ALT lungs exhibited a significantly greater rate of rise in pulmonary artery pressures for all hypoxic exposures compared to CON lungs.

**Responses to Acute Hypoxia/CO+Hypoxia Protocol B****Control Rats** (Figure 8)

In CON lungs, there were no significant differences in pressor responses to an hypoxic (6% CO<sub>2</sub>, balance N<sub>2</sub>) challenge at 5 min of exposure, compared to responses to a hypoxic gas mixture which contained CO (6.08% CO<sub>2</sub>, 945 ppm CO, balance N<sub>2</sub>).

**Altitude Rats** (Figure 9)

Pulmonary pressor responses to CO+HX were not significantly different than responses to HX-2, HX-3, or HX-4 in ALT lungs. The responses to HX-1 were significantly (P<0.05) less than HX-2, HX-3, and CO+HX. HX-4 responses were significantly smaller compared to responses of HX-3.

**Responses to AII During Hypoxia-Protocol B****Control Rats** (Figure 10)

There were no significant differences in CON lungs injected with AII (0.4μg) during exposure to hypoxia (6% CO<sub>2</sub>, balance N<sub>2</sub>) compared to responses to AII injections during exposure to a hypoxia and CO gas mixture (6% CO<sub>2</sub>, 945 ppm CO, balance N<sub>2</sub>). Responses to AII injections (0.4μg) (n=5) during hypoxic challenges were significantly (P<0.05) smaller than the final AII challenge (AII-5) which was given subsequent to the final hypoxic challenge (Figure 11).

**Altitude Rats** (Figure 12)

There were no significant differences in ALT isolated rat lungs injected with AII during exposure to hypoxia (6% CO<sub>2</sub>, balance N<sub>2</sub>) compared to responses to AII injections during exposure to a hypoxia and CO gas mixture (6% CO<sub>2</sub>, 945 ppm CO, balance N<sub>2</sub>). Responses to AII during hypoxia or CO plus hypoxia were not significantly different compared to AII responses between hypoxia and CO plus hypoxia exposures (Figure 13).

**Perfusate pH, PO<sub>2</sub>, and PCO<sub>2</sub>**

There were no significant differences in the perfusate pH, PO<sub>2</sub>, PCO<sub>2</sub>, between hypoxic and CO plus hypoxic gas exposures in the present study (Table 4).

Table 2. Heart weights and hematocrit in control and altitude-exposed rats.

---

	<u>RV/(LV+S)</u>	<u>RV/BW (mg/g)</u>	<u>HEMATOCRIT (%)</u>
CONTROL (n=16)	0.27 ± 0.01	0.52 ± 0.02	45.9 ± 0.8
ALTITUDE (n=12)	0.41 ± 0.01*	0.76 ± 0.02*	55.8 ± 1.2*

---

\* denotes different from CONTROL. P<0.05. Mean ± SE.

Table 3. Rates of rise and decline of pulmonary artery pressures in response to hypoxic and carbon monoxide plus hypoxic challenges (Protocol A).

MEASURE		RATE OF RISE (mm/sec)			RATE OF DECLINE (mm/sec)	
CHALLENGE	HX-2			HX-2		
					CO+HX	HX-3
CON (n=8)	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.001
ALT (n=6)	0.11 ± 0.03*	0.12 ± 0.02*	0.11 ± 0.02*	0.01 ± 0.004	0.02 ± 0.003	0.01 ± 0.002

"HX-2" denotes second hypoxic challenge. "CO+HX" denotes carbon monoxide plus hypoxic challenge. "HX-3" denotes third hypoxic challenge. \* denotes significantly greater than CONS. Mean ± SE. P<0.05.



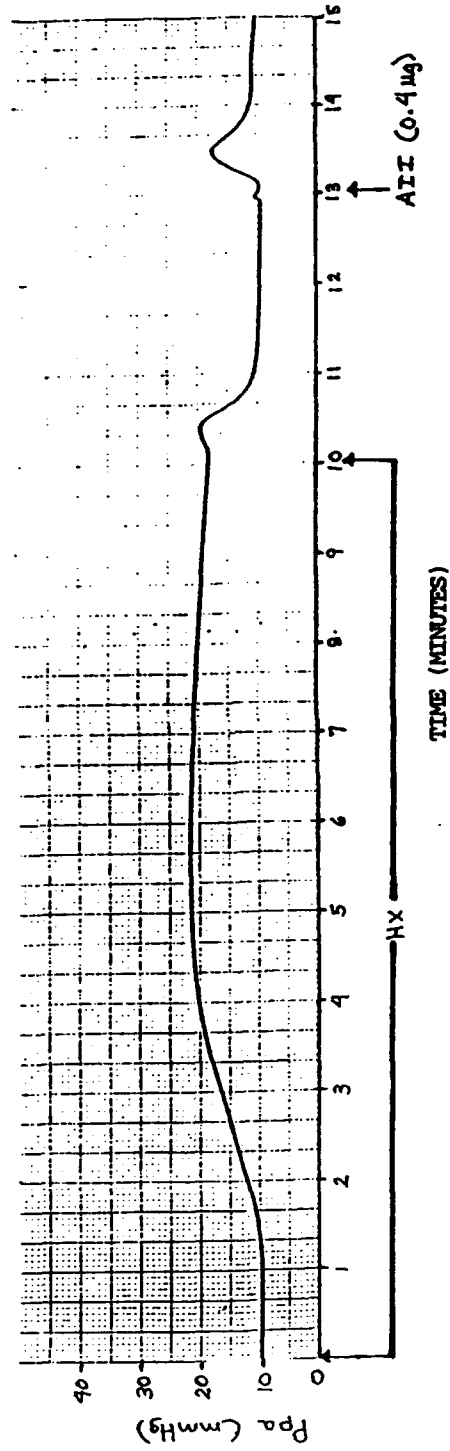


Figure 1. Pressure wave forms for acute hypoxia and angiotensin II challenges in isolated perfused rat lungs (Protocol A).

## HYPOXIA/CO+HYPOXIA-CONTROL RATS (PROTOCOL A)

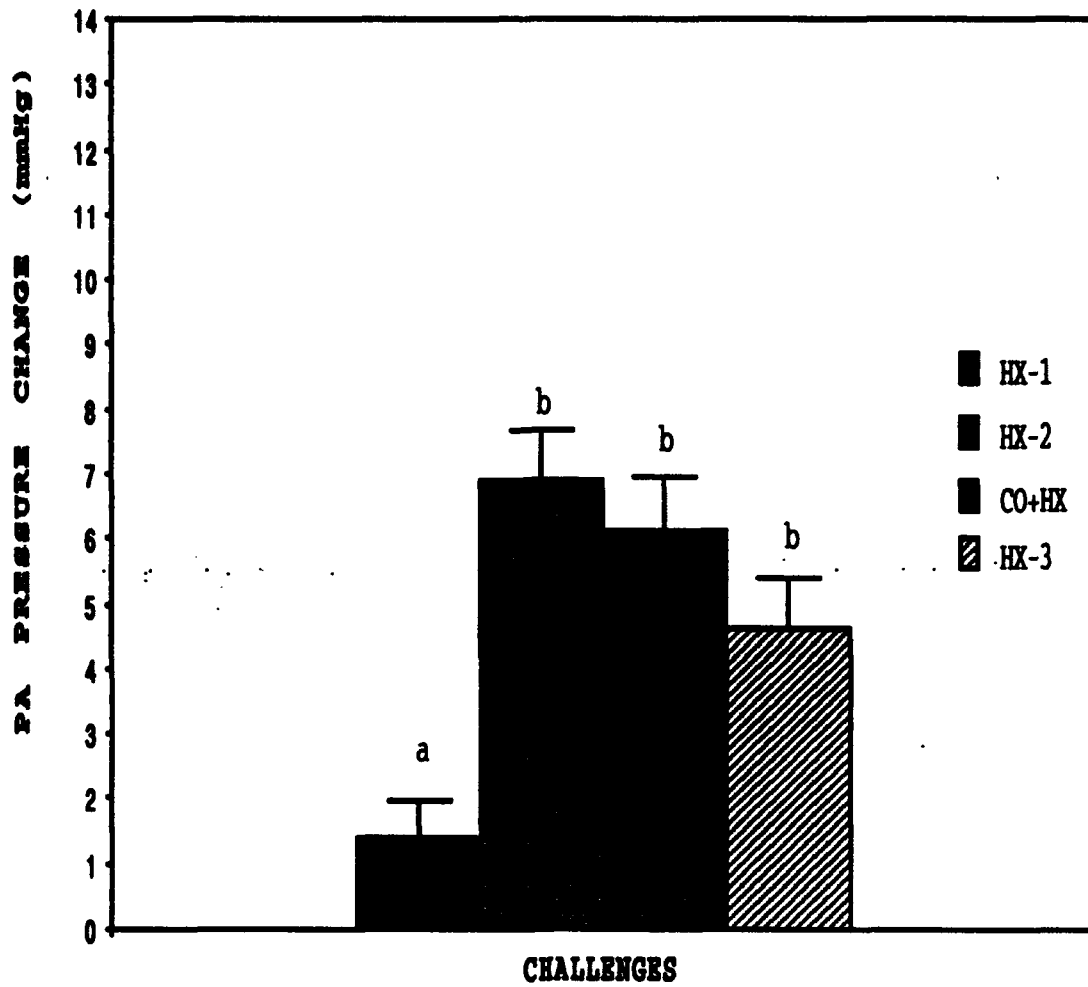


Figure 2. Peak pulmonary pressor responses to acute hypoxia in CON lungs (protocol A). a significantly less than b.  $P < 0.05$  Mean  $\pm$  SE. (n=8)

## HYPOXIA/CO+HYPOXIA-ALTITUDE RATS (PROTOCOL A)

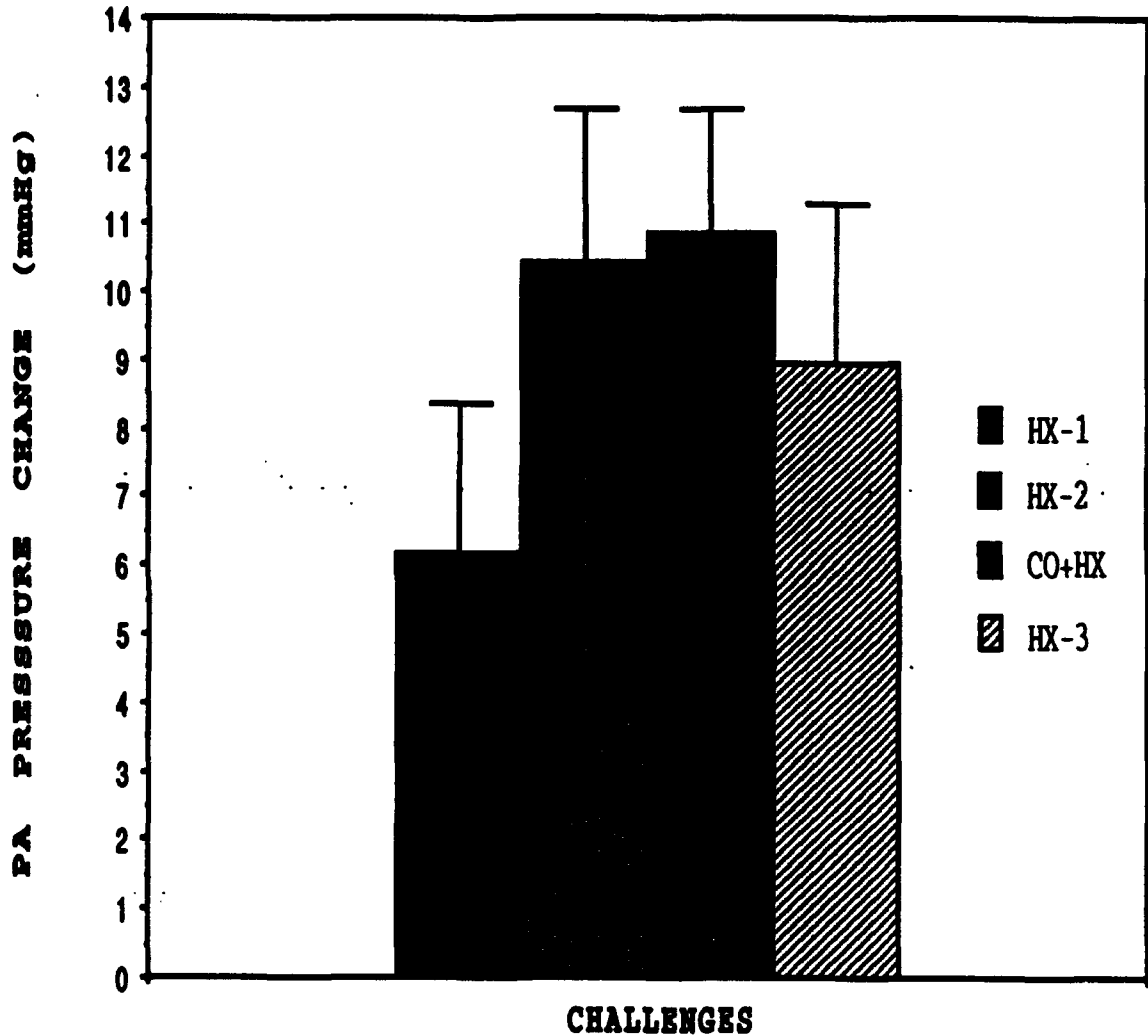


Figure 3.

Pulmonary pressor responses to acute hypoxia and acute carbon monoxide plus hypoxia in ALT lungs (protocol A). Mean  $\pm$  SE. No significant differences among challenges.

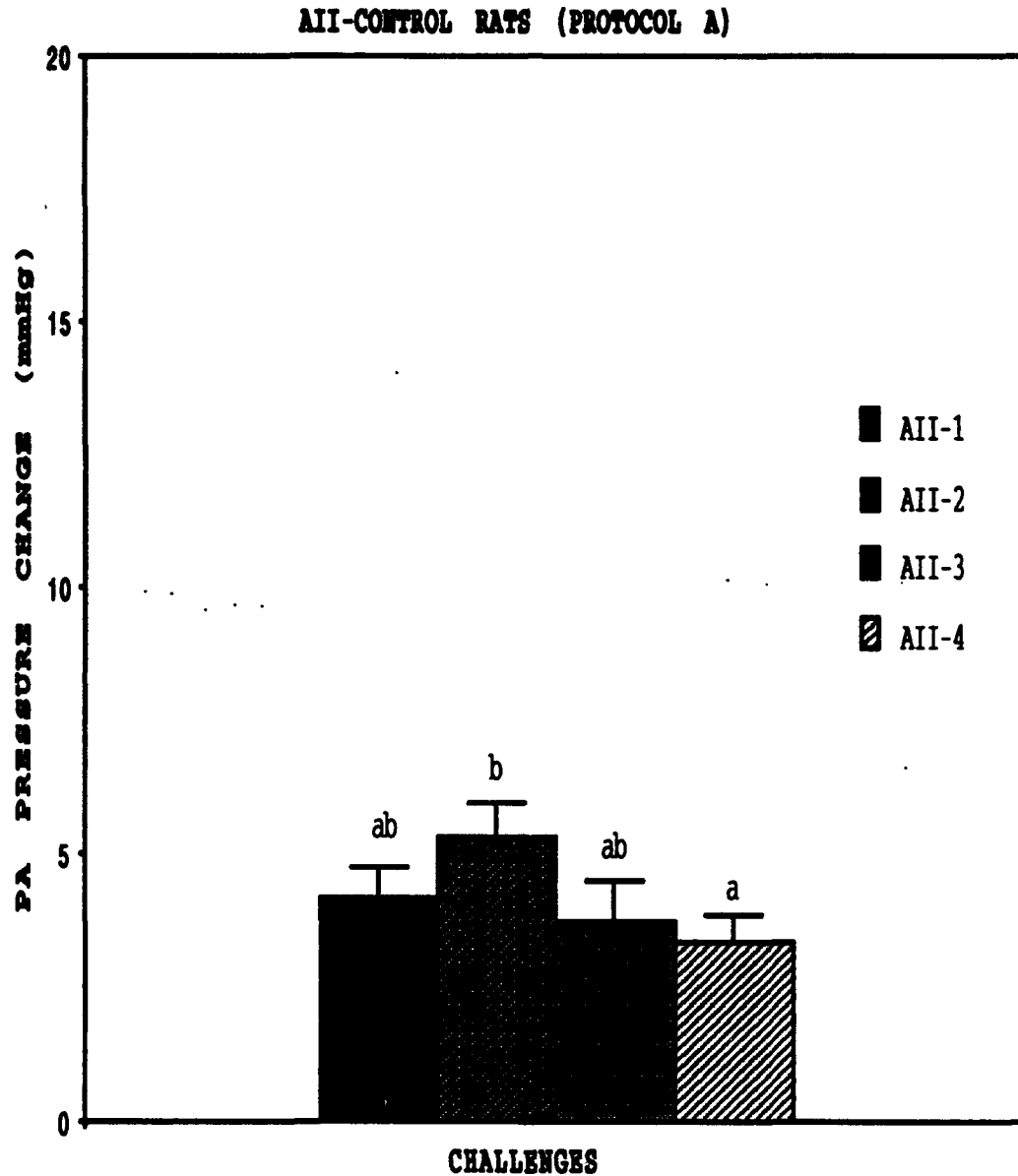


Figure 4. Pulmonary pressor responses to AII (0.4 $\mu$ g) in CON lungs (protocol A). The CO+HX challenge was given between AII-2 and AII-3. b significantly greater than a.  $P < 0.05$ . Mean  $\pm$  SE. (n=8)

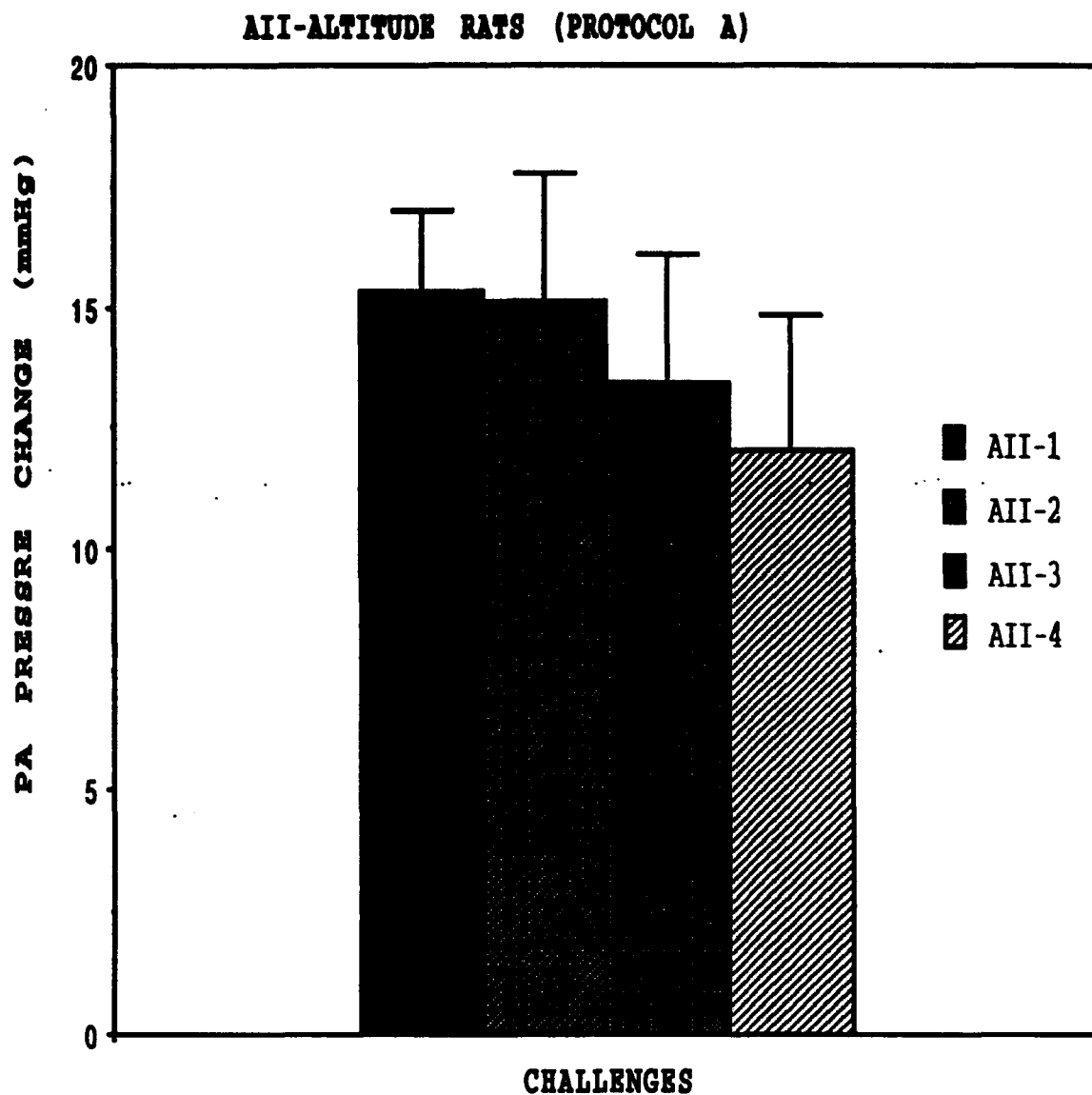


Figure 5. Pulmonary pressor responses to AII ( $0.4\mu\text{g}$ ) in ALT lungs (protocol A). The CO+HX challenge was given between AII-2 and AII-3. Mean  $\pm$  SE. No significant differences among challenges. (n=6)

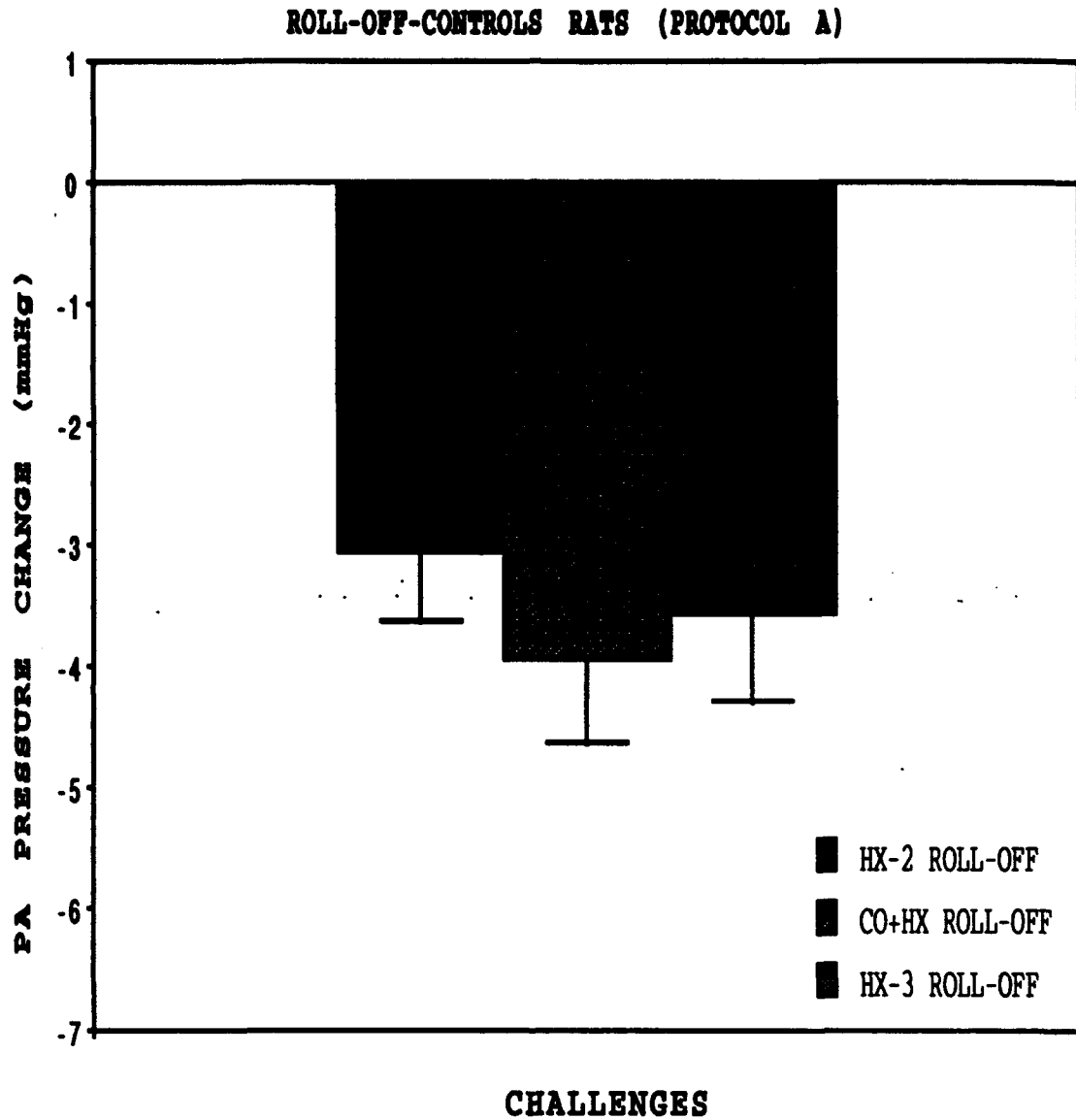


Figure 6. Pressure roll-off responses to acute hypoxia and CO plus hypoxia in CON lungs (protocol A). Mean  $\pm$  SE. No significant differences among challenges. (n=8)

## ROLL-OFF-ALTITUDE RATS (PROTOCOL A)

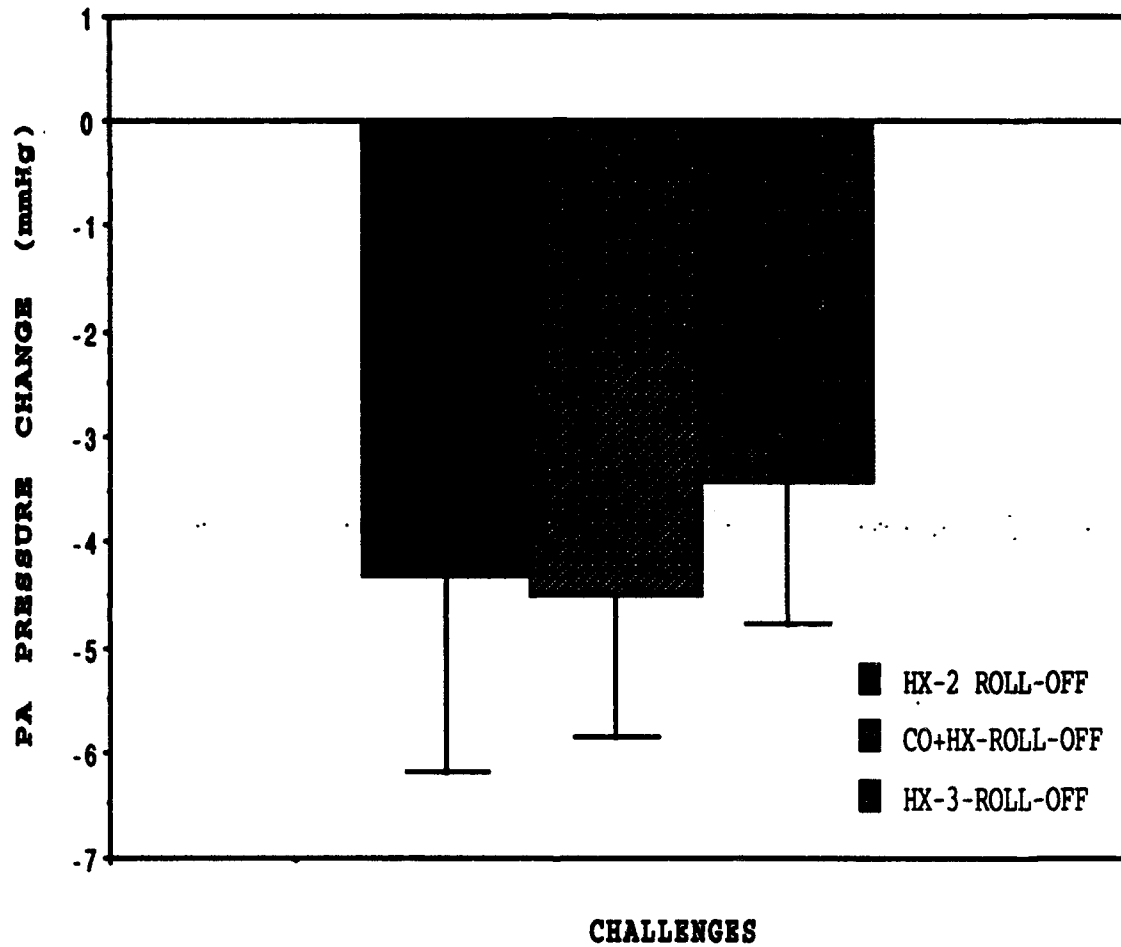


Figure 7. Pressure roll-off responses to acute hypoxia and acute carbon monoxide plus hypoxia in ALT lungs (protocol A). Mean  $\pm$  SE. No significant differences among challenges. (n=6)

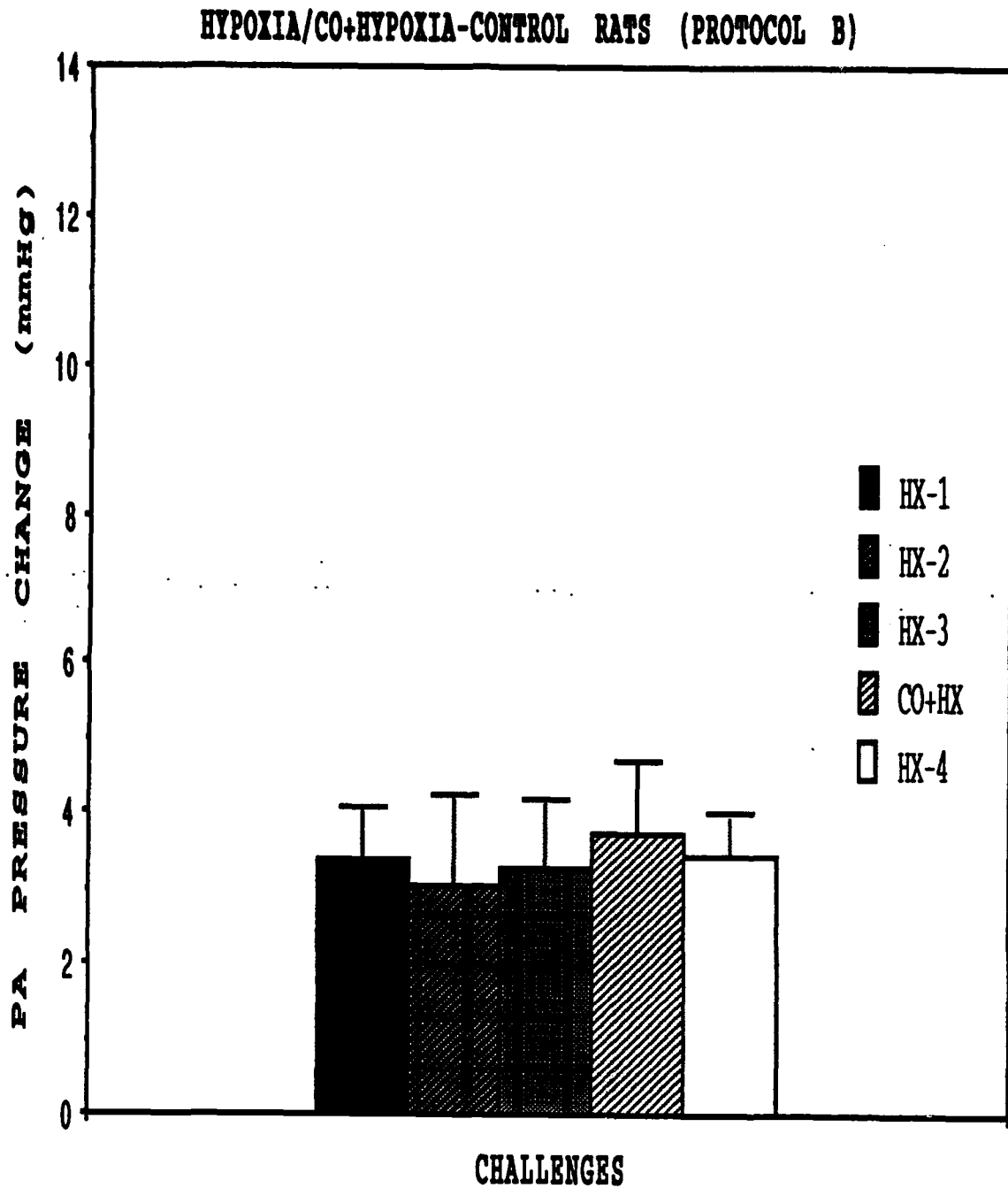


Figure 8. Pulmonary pressor responses to acute hypoxia and acute carbon monoxide plus hypoxia in CON lungs (protocol B). Mean  $\pm$  SE. No significant differences among challenges. (n=8)

## HYPOXIA/CO+HYPOXIA-ALTITUDE RATS (PROTOCOL B)

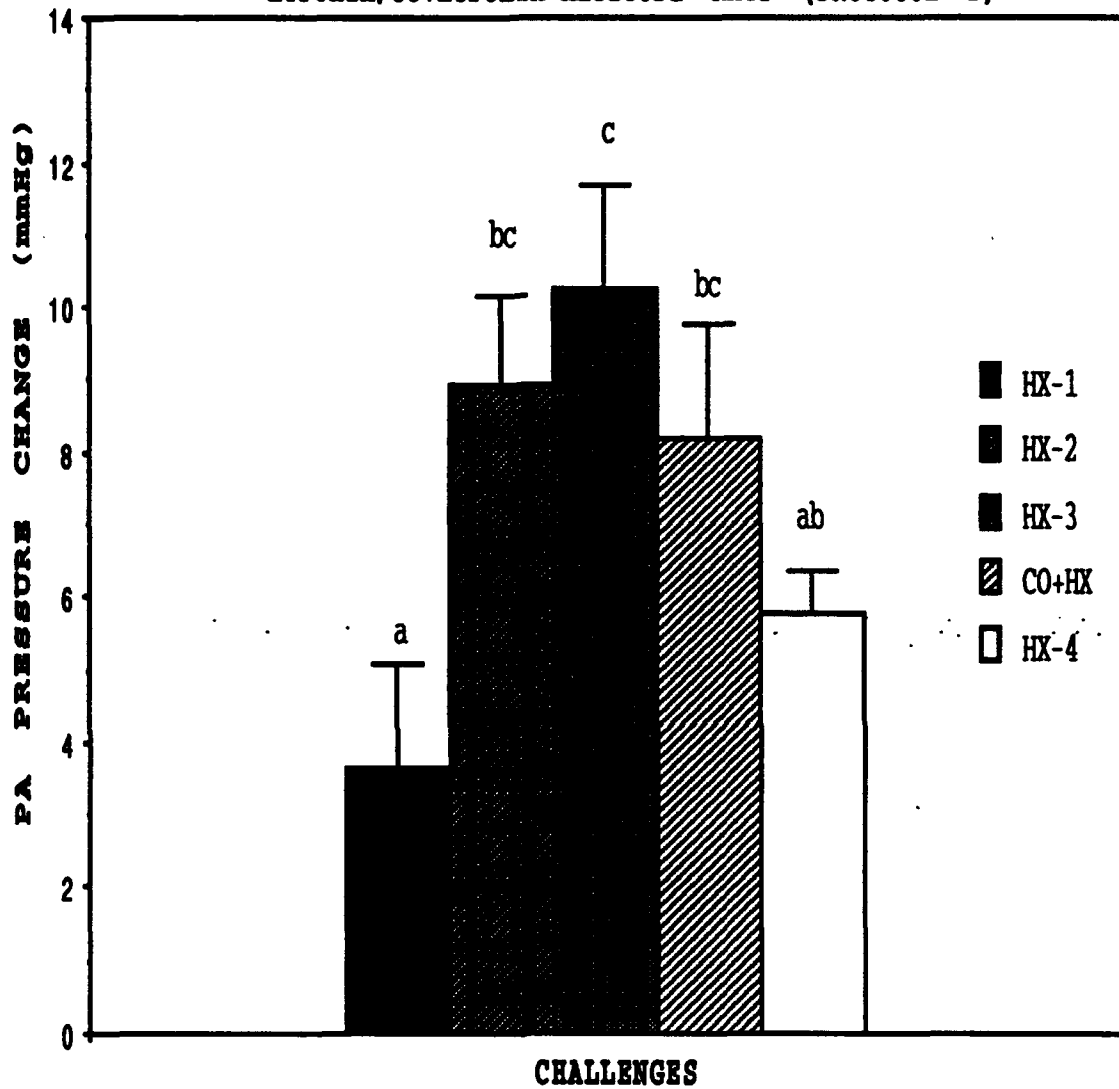


Figure 9.

Pulmonary pressor responses to acute hypoxia and acute carbon monoxide plus hypoxia in ALT lungs (protocol B). a significantly less than bc, c, and ab. c significantly greater than ab.  $P < 0.05$ . Mean  $\pm$  SE. (n=6)

## AII RESPONSES DURING HYPOXIA/CO+HYPOXIA-CON RATS (PROTOCOL B)

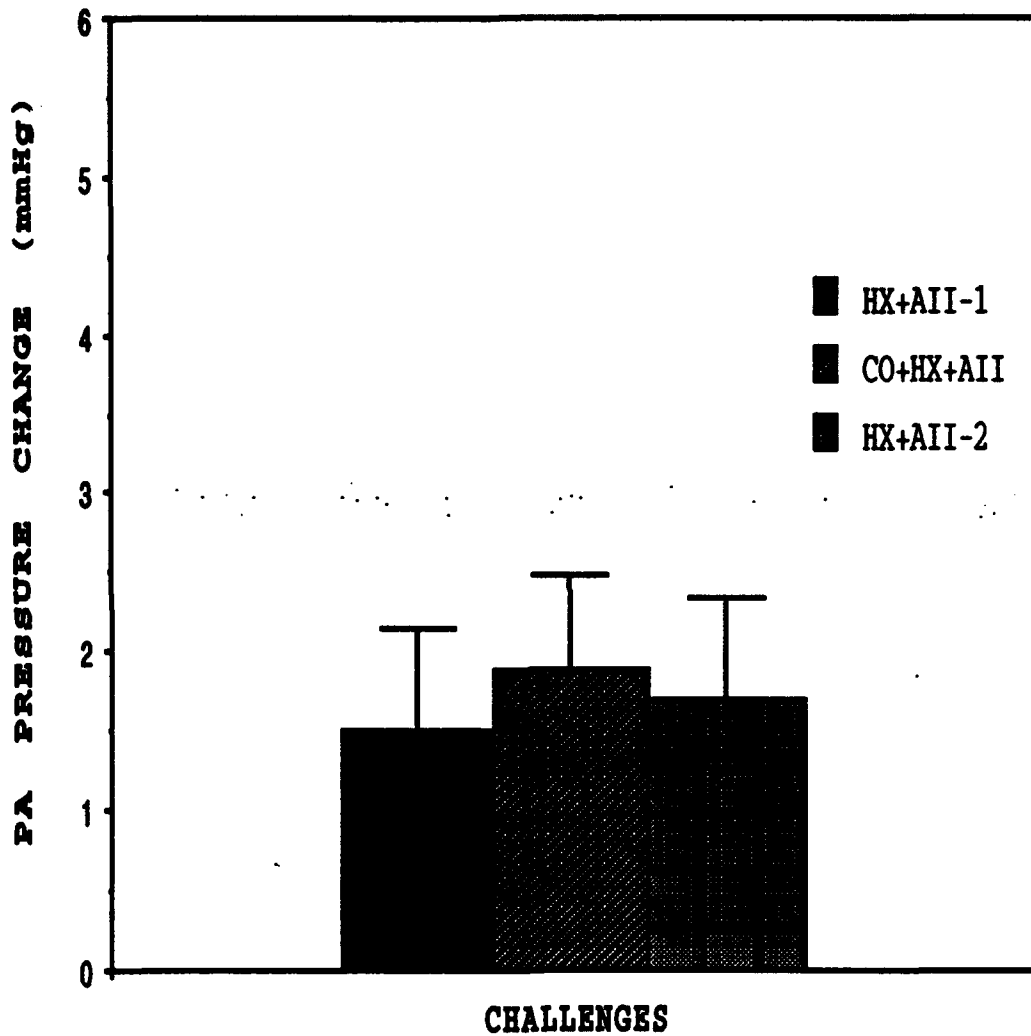


Figure 10. Pulmonary pressor responses to AII (0.4 $\mu$ g) during hypoxia and CO plus hypoxia in CON lungs (protocol B). Mean  $\pm$  SE. No significant differences among challenges. (n=5)

AII DURING AND BETWEEN HYPOXIA AND CO+HYPOXIA-CON RATS  
(PROTOCOL B)

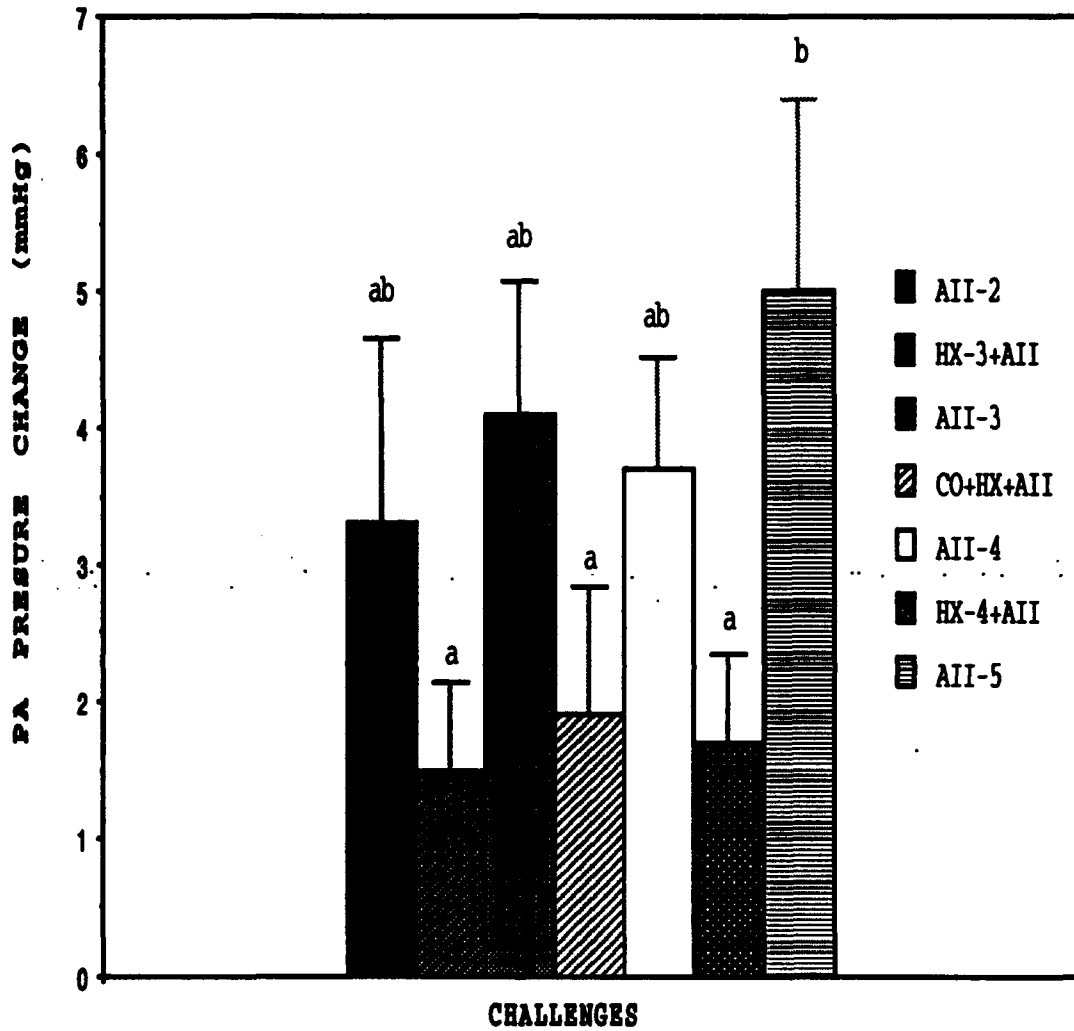


Figure 11. Pulmonary pressor responses to AII (0.4 $\mu$ g) between and during hypoxic challenges in CON lungs (protocol B). a significantly less than b.  $P < 0.05$ . Mean  $\pm$  SE (n=5)

## AII RESPONSES DURING HYPOXIA/CO+HYPOXIA-ALT RATS

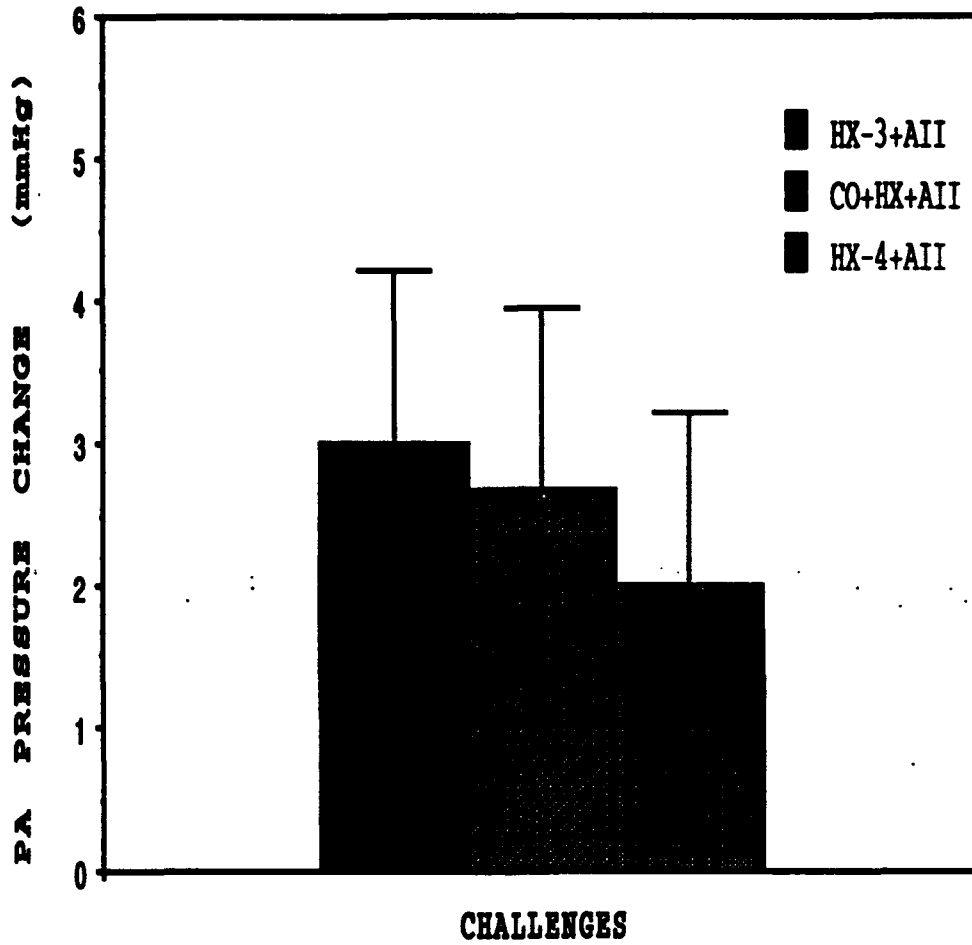


Figure 12. Pulmonary pressor responses to AII (0.2 μg) during hypoxia and CO plus hypoxia in ALT lungs (protocol B). Mean  $\pm$  SE. No significant differences among challenges. (n=6)

## AII DURING AND BETWEEN HYPOXIA AND CO+HYPOXIA-ALT RATS

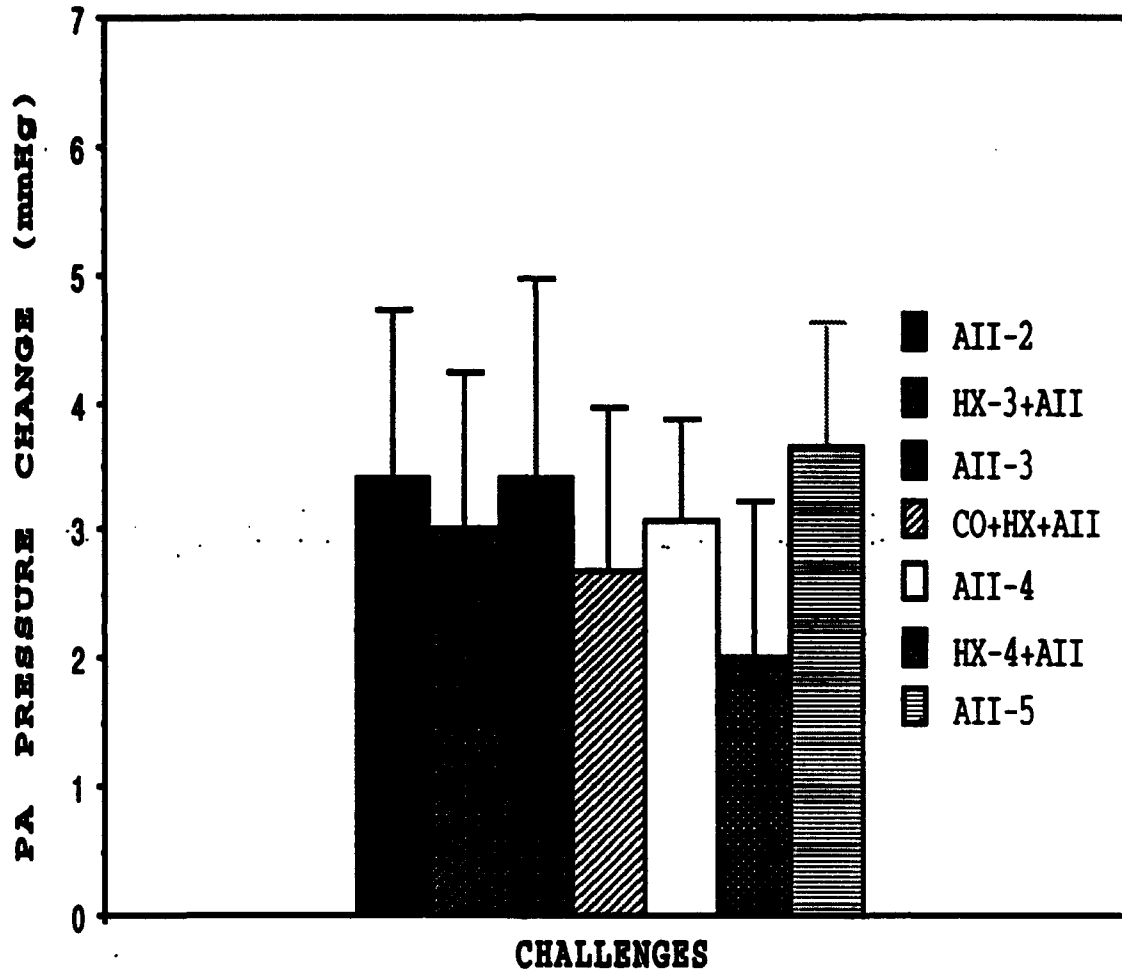


Figure 13. Pulmonary pressor responses to AII ( $0.2\mu\text{g}$ ) during and between hypoxia and CO plus hypoxia in ALT lungs (protocol B). Mean  $\pm$  SE. No significant differences among challenges. (n=6)

## **Chapter VI**

### **DISCUSSION**

In determining the appropriate protocols to employ to study our hypothesis, we relied on previous studies (22,23) conducted to determine pulmonary vascular reactivity in isolated rat lungs exposed to various challenges, including hypoxia and NO. Therefore, the two protocols were designed in such a manner as to allow each animal to serve as its own control by bracketing hypoxic challenges around the CO plus hypoxic challenges.

#### **Effects of Altitude on Heart Weights and Hematocrit**

RV/(LV+S) ratios, RV/BW ratios, and hematocrits were all increased in rats exposed to simulated high altitude compared to low altitude controls. Since these parameters are indirect indicators of pulmonary hypertension in rats (27), these observations confirm that the altitude-exposed rats of the present study were pulmonary hypertensive, and that the experiments did assess the effects of acute CO exposure on hypoxic pulmonary vasoconstriction in two distinct animal models (normotensive and pulmonary hypertensive).

## **Effects of Acute Carbon Monoxide Exposure on Pulmonary Vascular Reactivity**

Inhaled NO has recently been demonstrated to selectively dilate the pulmonary vasculature in patients with pulmonary hypertension (28-31), and in sheep with hypoxia-induced pulmonary hypertension (10). In addition, low concentrations (<100 ppm) of NO have been demonstrated to significantly

enuate increases in pulmonary artery pressure in response to hypoxia and AII challenges in isolated rat lungs (23). The reported physical and chemical similarities between NO and CO (17,32), combined with research demonstrating CO's ability to cause vasodilation in various species (21,33,34), led us to speculate that low concentrations of inhaled CO would also attenuate pulmonary pressor responses to hypoxia and AII in isolated salt-perfused rat lungs.

### Responses to Acute Hypoxia

We hypothesized that inhaled CO (200 and 1000 ppm) would modulate hypoxic pulmonary vasoconstriction (HPV) by relaxing pulmonary vascular smooth muscle, thereby decreasing the magnitude of the hypoxic response as well as affecting the time course of HPV. The data obtained did not support this hypothesis. In CON and ALT lungs, there were no significant differences between the peak pulmonary pressor responses to hypoxia compared to the pressor responses induced by CO plus hypoxia. Neither was there any measurable effect of CO on the rate of rise, rate of decline, or the magnitude of the roll-

offs in the pulmonary pressor responses to hypoxia. We can only speculate as to why, in the present study, CO had no apparent effect on HPV. It is possible that CO in concentrations greater than that used in the present study may be needed induce pulmonary vasodilation. McGrath and Smith demonstrated a vasodilatory response in isolated rat hearts perfused with a salt solution equilibrated with 10% CO (35). In studies investigating the ability of CO to relax other vascular smooth muscle in vitro (12,17,36-38), concentrations were as high as 2.5 to 5.0% CO, which is equivalent to 25,000 and 50,000 ppm, respectively. Since CO has been demonstrated to have effects on systemic vascular smooth muscle at these levels, it seems likely that similar CO concentrations may stimulate pulmonary vascular smooth muscle. The concentrations used in the present study (200 and 1000 ppm) were chosen because of similar studies conducted with NO (20 to 1,000 ppm), and studies on the effects of chronic exposure of rats to CO (200 and 500 ppm) (39,40). There are, to our knowledge, no studies which have investigated the effects acute inhaled CO on pulmonary vascular reactivity and therefore, further studies are needed to determine the levels of CO needed to alter the pulmonary vascular reactivity. However, using higher CO concentrations increases the risk of CO cytotoxicity (41,42), thereby possibly masking a physiological action.

The present study confirmed a previous report (22) that lungs of rats exposed to simulated high altitude for 4 to 6 weeks show increased pressor responses to hypoxic challenges. In both protocols, the peak hypoxic pulmonary pressor responses were larger in ALT lungs compared to CON lungs. It has also been shown that lungs of rats which have been chronically exposed to simulated high altitude have decreased pressor responses to hypoxic challenges for several hours after removal from simulated high altitude (43). Therefore, to avoid this phenomenon, in the present study the rats were removed from the hypobaric chamber 24 hrs prior to experimentation.

The present study was conducted using Earle's salt solution as the perfusate instead of whole blood. Since it is well known that CO has a very high affinity for hemoglobin, and is known to elicit physiological responses due to its interaction with heme proteins (15,44), we wanted to assess the effects of CO on the pulmonary circulation independent of its effects on hemoglobin. CO has been previously demonstrated to have effects on tissues independent of those associated with carboxyhemoglobin (42). We speculated that CO would diffuse across the the pulmonary capillary membranes, dissolve in the perfusate, and be available in the solution as free CO to potentially influence the pulmonary circulation. Bassett and Fisher (41), using isolated salt-perfused rat lungs exposed to inhaled CO (95% CO 5% CO<sub>2</sub>),

demonstrated the ability of CO to significantly alter lung cellular metabolism. Other studies have demonstrated that CO dissolved in solution void of heme proteins has the ability to induce cellular changes and relax smooth muscle tissue (11,35,45,46). In the present study, the perfusate was not analyzed to determine the amount of dissolved CO, however, the estimated  $P_{CO}$  in perfusate (based on 1000 ppm CO,  $P_B = 640$  mmHg) was 0.65 mmHg.

Although CO has been studied for many years, there is at present no study which has measured the amounts of endogenous CO produced by various tissues in order to determine whether CO is produced in vivo in sufficient amounts to produce physiological responses. Likewise, the data on combined CO and altitude exposure is also limited. The studies by McGrath, Cooper, and Davies (47-51) are, to our knowledge, the only investigations into the effects of combined altitude and CO exposure in animals. In these studies, carboxyhemoglobin levels were elevated in rats exposed to high altitude, suggesting increased endogenous CO production. This increase in endogenous CO is reportedly due to increased heme catabolism as a result of altitude-induced polycythemia. Is it possible that endogenous CO may have a physiological role in response to high altitude? The present study did not specifically address this question and therefore, further research is needed in this area.

In summary, the results of the present study do not support the hypothesis that CO modulates the magnitude or the time course of HPV by relaxing pulmonary vascular smooth muscle in isolated salt-perfused rat lungs. Although previous studies have demonstrated the ability of CO dissolved in solutions other than blood to relax smooth muscle, we were unable to verify that CO (200 and 1000 ppm) was in the perfusate in high enough concentrations to elicit a response. In addition, since there is very little data on CO exposure and the pulmonary vasculature, we can only speculate that greater concentrations of CO (25,000 to 50,000 ppm) are needed to elicit a physiological response in the pulmonary vasculature of the rat.

#### Responses to Angiotensin II

We hypothesized that inhaled CO would attenuate the pulmonary pressor response to angiotensin II in isolated rat lungs. In order to test this hypothesis we injected AII during hypoxic and CO plus hypoxic challenges. In both CON and ALT lungs, CO did not alter the pulmonary pressor response to AII. However, since the experimental set-up used in this study did not permit us to assess the effects of CO alone on AII-induced vasoconstriction, and although there were also no significant differences in pulmonary pressor responses to AII injections given between hypoxic and CO plus hypoxic challenges, we cannot be certain that CO alone, does not affect pulmonary pressor responses to AII. In a

similar study involving NO and its effects on AII-induced vasoconstriction, NO did significantly attenuate the pressor response (23). Since NO and CO are presently suggested to relax vascular smooth muscle via the same mechanism (i.e. by regulating intracellular cGMP levels), we can only speculate that either the levels of CO used in this present study were not sufficient to activate guanylate cyclase and increase cellular levels of cGMP in the pulmonary vasculature, or, NO attenuates pressor responses to AII via a mechanism other than cGMP. To our knowledge, there have been no studies to determine cGMP levels in isolated rat lungs exposed to hypoxic or AII-induced vasoconstriction to determine whether the cellular levels are modified.

Our data support that of previous research which demonstrated that lungs of rats chronically exposed to simulated high altitude have increased pulmonary pressor responses to AII (43). The ALT lungs in protocol A showed increased reactivity to AII (0.4 $\mu$ g) injections compared to similarly challenged CON lungs.

The pressor responses to AII given during a hypoxic or CO plus hypoxic exposure were decreased in both ALT and CON lungs compared to the AII responses given between hypoxic challenges. In addition, the pulmonary pressor response was maintained for a longer period of time when AII was injected during an hypoxic challenge. This result confirms findings of Voelkel et al. (52), who showed delayed vasodilation time in

isolated rat lungs given AII injections during hypoxia. The reason for this decreased AII-response during hypoxia is not clear. It has been suggested that hypoxia may slow the metabolism of angiotensin or possibly inhibit the degradation of other locally produced vasoconstrictor substances such as serotonin (52). In both protocols, the magnitude of the AII-induced vasoconstriction diminished with repeated AII injections, confirming previous findings (53). It is unlikely that this diminished responsiveness masked a physiological effect of CO, due to the fact that there was no significant attenuation of the AII response given during CO plus hypoxia. Therefore, this decrease in responsiveness was most likely due to tachyphylaxis (54).

In summary, CO did not attenuate the pulmonary pressor response induced by injections of angiotensin during CO plus hypoxia. Neither did it attenuate AII-induced pressure responses subsequent to hypoxic challenges.

#### **Perfusate pH, PO<sub>2</sub>, and PCO<sub>2</sub>**

It has been reported that alterations in perfusate pH, PO<sub>2</sub>, and PCO<sub>2</sub> can affect the degree of HPV in isolated lungs (55-57). In the present study, there were no significant differences in perfusate pH, PO<sub>2</sub>, and PCO<sub>2</sub> between hypoxic and CO plus hypoxic challenges. Therefore, any differences in hypoxic pressor responses should have reflected the effects of CO on HPV.

## CHAPTER VII

### SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

#### SUMMARY

This study investigated the role of carbon monoxide (CO) in modulating hypoxic pulmonary vasoconstriction (HPV) in the isolated salt-perfused rat lung. Although the underlying mechanism of HPV remains unknown, recent research has focused on endogenous vasodilators such as nitric oxide (NO) as having a major role in modulation of pulmonary vascular smooth muscle tone in normal and hypoxic lungs. CO, another reported smooth muscle vasodilator, is also endogenously produced and has been shown to have actions similar to those of NO in both animals and humans. Therefore, we investigated the effects of acute CO exposure on the hypoxic and angiotensin II-induced vasopressor responses in the isolated salt-perfused rat lung model. Pulmonary hypertensive (produced by exposure to simulated high altitude) rats were also used to determine the effects of acute CO exposure in a remodeled pulmonary vascular bed.

**Conclusions**

In the present study, it was demonstrated that in the isolated salt-perfused rat lung, acute exposure to low concentrations of carbon monoxide (<1000 ppm), did not significantly alter either the magnitude or the time-course of the hypoxic pressor response in either control or altitude-exposed rats. In addition, CO did not affect the pulmonary pressor responses to angiotensin II, either given between, or during hypoxic or hypoxic plus CO challenges. Therefore, we conclude that CO, in concentrations less than 1000 ppm does not modulate pulmonary vascular reactivity in lungs isolated from control or altitude-exposed isolated rats.

**Recommendations**

To confirm or challenge the information presented in this study, the following studies are recommended:

1. A similar study should be conducted using increasing concentrations of CO (>1000 ppm) in order to determine the concentration of CO required to elicit a response from the pulmonary vasculature. We speculate that since CO (2.5 to 5%) has been shown to relax vascular smooth muscle in tissues other than pulmonary vascular smooth muscle, exposing the lungs to similar inhaled CO concentrations would relax pulmonary smooth muscle and therefore attenuate hypoxic pulmonary pressor response in isolated rat lungs.

2. A similar study should be conducted using blood as the perfusate. A study investigating the effects perfusate solutions on the actions of NO showed decreased attenuation of the hypoxic pressor response in blood-perfused rat lungs compared to similarly treated salt-perfused rat lungs. Since NO and CO both have high affinities for hemoglobin, we speculate that in blood perfused rat lungs, hemoglobin would act as a "sink" for CO and therefore decrease its ability to act directly on the pulmonary vasculature.
3. A similar study should be conducted to determine levels of cyclic GMP during CO and hypoxic exposures. Since CO is thought to relax vascular smooth muscle by increasing levels of cGMP, perfusate or tissue samples could be analyzed, using radioimmunoassay, to determine whether CO alters cGMP levels in the pulmonary vasculature.
4. A study should be conducted to determine effects of low concentrations of CO on the responsiveness of isolated pulmonary arteries. To our knowledge, specific actions of CO on isolated pulmonary blood vessels have not been investigated.

## REFERENCES

1. BEST AND TAYLOR'S Physiological Basis of Medical Practice, Baltimore:Williams & Wilkins, 1991. 12th Ed. pp. 529-537.
2. Cutaia, M. and Rounds, S. Hypoxic pulmonary vasoconstriction. Physiologic significance, mechanism, and clinical relevance. Chest 97:706-718, 1990.
3. Levitzky, M.G. Pulmonary Physiology, New York:McGRAW-HILL, INC., 1991. 3rd Ed. pp. 100-101.
4. Penney, D.G., Tucker, A. and Bambach, G.A. Heart and lung alterations in neonatal rats exposed to CO or high altitude. J.Appl.Physiol. 73:1713-1719, 1992.
5. Kennedy, T.P., Michael, J.R. and Summer, W. Calcium channel blockers in hypoxic pulmonary hypertension. Am.J.Med. 78(suppl 2B):18-26, 1985.
6. Von Euler, U.S. and Liljestrand, G. Observations on the pulmonary arterial blood pressure in the cat. Acta Physiol.Scand. 12:301-320, 1946.
7. Voelkel, N.F. Mechanisms of hypoxic pulmonary vasoconstriction. Am.Rev.Respir.Dis. 133:1186-1195, 1986.
8. Farrukh, I.S. and Michael, J.R. Cellular mechanisms that control pulmonary vascular tone during hypoxia and normoxia. Possible role of Ca<sup>2+</sup> ATPases. Am.Rev.Respir.Dis. 145:1389-1397, 1992.
9. Palmer, R.M.J., Ferrige, A. G. and Moncada, S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524-526, 1987.
10. Pison, U., Lopez, F.A., Heidelmeyer, C.F., Rossaint, R. and Falke, K.J. Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction without impairing gas exchange. J.Appl.Physiol. 74:1287-1292, 1993.

11. Ramos, K.S., Lin, H. and McGrath, J.J. Modulation of cyclic guanosine monophosphate levels in cultured aortic smooth muscle cells by carbon monoxide. Biochem.Pharmacol. 38:1368-1370, 1989.
12. Lin, H. and McGrath, J.J. Carbon monoxide effects on calcium levels in vascular smooth muscle. Life.Sci. 43:1813-1816, 1988.
13. Carbon Monoxide and Human Lethality: Fire and Non-Fire Studies, London:Elsevier Applied Science LTD, 1993. pp. 3-60.
14. Haldane, J.S. The action of carbonic oxide on man. J.Physiol. 18:402-462, 1895.
15. Penney, D.G. A review: hemodynamic response to carbon Monoxide. Environ.Health Perspect. 77:121-130, 1988.
16. Farrugia, G., Irons, W.A., Rae, J.L., Sarr, M.G. and Szurszewski, J.H. Activation of whole cell currents in isolated human jejunal circular smooth muscle cells by carbon monoxide. Am.J.Physiol.Gastrointest.Liver Physiol. 264:G1184-G1189, 1993.
17. Schmidt, H.H.H.W. NO, CO, and OH endogenous soluble guanylyl cyclase-activating factors. FEBS Lett. 307, No 1:102-107, 1992.
18. Mianes, M. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J. 2:2557-2568, 1988.
19. Tremblay, J., Gerzer, R. and Hamet, P. Cyclic GMP in Cell Function . In: Advances in Second Messenger and Phosphoprotein Research, edited by Greengard, P. and Robison, G.A. New York: Raven Press, vol.22, 1988, p. 319-383.
20. Furchgott, R.F. and Jothianandan, D. Endothelium-dependent and independent vasodilation involving cyclic GMP: relaxation induced by nitric oxide, carbon monoxide and light. Blood Vessels 28:52-61, 1991.
21. Duke, H. and Killick, E. Pulmonary vasomotor responses of isolated perfused cat lungs to anoxia. J.Physiol. 117:303-316, 1952.
22. Tucker, A. and Penney, D.G. Pulmonary vascular responsiveness in rats exposed to high altitude or carbon monoxide. Exp.Lung.Res. 19:699-713, 1993.

23. Rich, G.F., Roos, C.M., Anderson, S.M., Ulrich, D.C., Daugherty, M.O. and John, R.A. Inhaled nitric oxide: dose response and the effects of blood in the isolated rat lung. J. Appl. Physiol. 75:1278-1284, 1993.
24. Grover, R.F. Pulmonary circulation in animals and man at high altitude. Ann. NY Acad. Sci. 127:632-639, 1965.
25. Tucker, A., McMurtry, L.F., Reeves, J.T., Alexander, A.F., Will, D.H. and Grover, R.F. Lung vascular smooth muscle as a determinant of pulmonary hypertension at high altitude. Am. J. Physiol. 228:762-767, 1975.
26. Stenmark, K.R., Fasules, J., Hyde, D.M., Voelkel, N.F., Henson, J., Tucker, A., Wilson, H., Reeves, J.T. Severe pulmonary hypertension and arterial adventitial changes in newborn calves at 4,300 m. J. Appl. Physiol. 62:821-830, 1987.
27. Hunter, C., Barber, G.R., Shaw, J.W. and Clegg, E.G. Growth of the heart and lungs in hypoxic rodents: a model of human hypoxic disease. Clin. Sci. Mol. Med. 46:375-391, 1974.
28. Kinsella, J.P. and Abman, S.H. Inhalational nitric oxide therapy for persistent pulmonary hypertension of the newborn. Pediatrics 91:997-998, 1993.
29. Weitzberg, E., Rudehill, A. and Lundberg, J.M. Nitric oxide inhalation attenuates pulmonary hypertension and improves gas exchange in endotoxin shock. Eur. J. Pharmacol. 233:85-94, 1993.
30. Tonz, M., von Segesser, L.K. and Turina, M. Selective pulmonary vasodilatation with inhaled nitric oxide. J. Thorac. Cardiovasc. Surg. 105:760-762, 1993.
31. Frostell, C.G., Fratacci, M.D., Wain, J.C., Jones, R. and Zapol, W.M. Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 83:2038-2047, 1991.
32. Snyder, S.H. Nitric Oxide: First in a New Class of Neurotransmitters? Science 257:494-496, 1992.
33. Marshall, C., Cooper, D.Y. and Marshall, B.E. Reduced availability of energy initiates pulmonary vasoconstriction. Proc. Soc. Exp. Biol. Med. 187:282-286, 1988.
34. Sylvester, J.T. and McGowan, C. The effects of agents that bind to cytochrome P-450 on hypoxic pulmonary vasoconstriction. Circ. Res. 43:429-437, 1978.

35. McGrath, J.J. and Smith, D.L. Response of rat coronary circulation to carbon monoxide and nitrogen hypoxia. Proc. Soc. Exp. Biol. Med. 177:132-136, 1984.
36. McFaul, S. and McGrath, J.J. Studies on the mechanism of carbon monoxide-induced vasodilation in the isolated perfused rat heart. Toxicol. Appl. Pharmacol. 464-473, 1987.
37. Lin, H. and McGrath, J.J. Carbon monoxide effects on calcium levels in vascular smooth muscle. Life Sci. 43: 1813-1816, 1988.
38. Lin, H. and McGrath, J.J. Vasodilating effects of carbon monoxide. Drug. Chem. Toxicol. 11:371-385, 1988.
39. Clubb, F.J., Penney, D.G. and Bishop, S.P. Cardiomegaly in neonatal rats exposed to 500 ppm carbon monoxide. J. Mol. Cell. Cardiol. 21:945-955, 1989.
40. Clubb, F.J., Penney, D.G., Baylerian, M. S. and Bishop, S. P. Cardiomegaly due to myocyte hyperplasia in perinatal rats exposed to 200ppm carbon monoxide. J. Mol. Cell. Cardiol. 18:477-486, 1986.
41. Bassett, D.J.P. and Fisher, A.B. Metabolic responses to carbon monoxide by isolated rat lungs. Am. J. Physiol. 230:658-663, 1976.
42. Golbaum, L.R., Ramirez, R.G. and Absalon, K. What is the mechanism of carbon monoxide toxicity? Aviat. Space Environ. Med. 46:1289-1291, 1975.
43. McMurtry, I.F., Petrun, M.D. and Reeves, J.T. Lungs from chronically hypoxic rats have decreased pressor response to acute hypoxia. Am. J. Physiol. 235:H104-H109, 1978.
44. Dodds, R.G., Penney, D.G. and Sutariya, B.B. Cardiovascular, metabolic, and neurologic effects of carbon monoxide and cyanide in the rat. Toxicol. Lett. 61:243-254, 1992.
45. Trischmann, U., Klockner, U., Isenberg, G., Utz, J. and Ullrich, V. Carbon monoxide inhibits depolarization-induced Ca rise and increases cyclic GMP in visceral smooth muscle cells. Biochem. Pharmacol. 41:237-241, 1991.
46. Utz, J. and Ullrich, V. Carbon monoxide relaxes ileal smooth muscle through activation of guanylate cyclase. Biochem. Pharmacol. 41:1195-1201, 1991.

47. McGrath, J.J. Effects of altitude on endogenous carboxyhemoglobin levels. J.Toxicol.Environ.Health 35:127-133, 1992.
48. Davies, D.G. and McGrath, J.J. Arterial blood gas status in rats exposed to chronic CO at low and high altitude. Respir.Physiol. 75:193-198, 1989.
49. McGrath, J.J. Cardiovascular effects of chronic carbon monoxide and high- altitude exposure. Res.Rep.Health Eff.Inst. 1-23, 1989.
50. Cooper, R., McGrath, J.J., Dooley, S. and Kopetzky, M.T. Chronic exposure to carbon monoxide at high altitude: effects on mean electrical axis. Physiol.Behav. 46:75-79, 1989.
51. McGrath, J.J. Carbon monoxide studies at high altitude. Neurosci.Biobehav.Rev. 12:311-314, 1988.
52. Voelkel, N.F., McMurtry, I.F. and Reeves, J.T. Hypoxia impairs vasodilation in the lung. J.Clin.Invest. 67:238-246, 1981.
53. Eugenio, F.N., Silva, E., Paiva, T. and Paiva, A. Increased intracellular Na<sup>+</sup> and depolarization favor angiotensin tachyphylaxis in rabbit aorta. Am.J.Physiol. 260:H373-H378, 1991.
54. Vicaut, E., Hou, M., Stucker, O. and Teisseire, B. Arteriolar vasoconstriction and tachyphylaxis with intraarterial angiotensin II. Microvasc.Res. 37:28-41, 1989.
55. Marshall, C., Lindgren, L. and Marshall, B.E. Metabolic and respiratory hydrogen ion effects on hypoxic pulmonary ventilation. J.Appl.Physiol. 57:545-550, 1984.
56. Marshall, C. and Marshall, B.E. Influence of perfusate PO<sub>2</sub> on hypoxic pulmonary vasoconstriction in rats. Circ.Res. 52:691-696, 1983.
57. Hyde, R.W., Lawson, W.H. and Forster, R.E. Influence of carbon dioxide on the pulmonary vasculature. J.Appl.Physiol. 19:734-744, 1964.