HYPOCAPNIA-INDUCED BRONCHOCONSTRICTION

By

Ann Michelle Reynolds, B.Sc.

A THESIS SUBMITTED FOR THE DEGREE OF

MASTER OF SCIENCE

Department of Physiology

The University of Adelaide

and

Department of Thoracic Medicine

Royal Adelaide Hospital

South Australia

September 1988

Dedicated to

my

Mother and Father

TABLE OF CONTENTS

Page

SUMMARY

Hypocapnia-induced bronchoconstriction has been recognized for over 30 years, however, its mechanisms have to date remained essentially unknown. This thesis describes a guinea pig model of hypocapnia-induced bronchoconstriction and elucidates the mechanisms involved in this airway response.

Despite the extensive use of guinea-pig isolated-lung preparations over past decades, severe postmortem bronchoconstriction in this species has only recently been described and has been observed in the preliminary studies of the present work reported herein. This phenomenon was re-examined by measuring postmortem airway function in anaesthetized open-chest guinea pigs following circulatory arrest, in an attempt to determine the initiating factors. The intensity of bronchoconstriction was assessed by calculating changes in dynamic compliance and measuring relaxation lung volume at the completion of the experiments. From these studies it was found that postmortem bronchoconstriction was principally due to airway hypocapnia, a known cause of bronchoconstriction. Changes in airway function were also observed if there was marked airway cooling and drying.

Following the establishment of this guinea pig model of hypocapnia-induced bronchoconstriction, a second detailed study was undertaken to determine what mediators were involved.

Tachykinins (a group of neuropeptides with a similar amino acid sequence at the C-terminal end) had recently been implicated in mediating guinea pig postmortem bronchoconstriction, thus raising the possibility that tachykinins may mediate the hypocapnia-induced bronchoconstriction observed in this species. This second study was designed to determine whether hypocapnia causes bronchoconstriction by releasing tachykinins from C-afferent nerves in airways. Three experimental interventions were used: 1) depletion of tachykinins by repeated capsaicin injections, 2) treatment with phosphoramidon, an inhibitor of enkephalinase, the main enzyme responsible for tachykinin inactivation, and 3) topical airway anaesthesia. Capsaicin pretreatment markedly attenuated the hypocapnia-induced changes in dynamic compliance and relaxation lung volume whereas phosphoramidon augmented these changes. Topical anaesthesia of airways with bupivacaine almost completely prevented hypocapnia-induced bronchoconstriction.

These studies demonstrate that in the guinea pig, postmortem bronchoconstriction is triggered by airway hypocapnia, and that this hypocapnia-induced bronchoconstriction is mediated by tachykinins which are released following the activation of bronchial axonal reflexes.

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any degree or diploma in any university, and to the best of my knowledge contains no material previously published by another person, except where due reference is made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Ann Michelle Reynolds

PUBLICATIONS

The following is a list of publications that have arisen from this work.

REYNOLDS A. M. AND McEVOY R. D. Prevention of abnormal pulmonary mechanics in the postmortem guinea pig lung. Thorax. 1987, 42:741. (Abstract)

REYNOLDS A. M. AND McEVOY R. D. Tachykinins mediate hypocapnia-induced bronchoconstriction (HIBC) in postmortem guinea pig lungs. Aust. N.Z. J. Med. 1988, 18:537. (Abstract)

REYNOLDS A. M. AND McEVOY R. D. Prevention of abnormal pulmonary mechanics in the postmortem guinea pig lung. J. Appl. Physiol. 1988, 64:1322-1326.

ACKNOWLEDGEMENTS

This thesis was undertaken with the supervision of Dr's R.D. McEvoy and T.S. Miles. I am deeply indebted to Dr. R. Doug McEvoy for his unfailing advice, encouragement and understanding without which this thesis would not have been possible.

I wish also to thank the staff of the Departments of Physiology (University of Adelaide) and Thoracic Medicine (Royal Adelaide Hospital) for providing me with laboratory space and facilities for this study.

I am grateful to Ms. D. Caine for her assistance in preparing several diagrams.

Especially, I wish to thank my parents for their ecouragement and support throughout my academic studies.

The author was the recipient of a Dawes, Curren, Hughes Research Fellowship awarded by the Royal Adelaide Hospital.

ABBREVIATIONS

A list of abbreviations used in this thesis.

ACh	Acetylcholine
Cdyn	Dynamic compliance
HIBC	Hypocapnic-induced bronchoconstriction
$P_{aw}CO_2$	Airway carbon dioxide partial pressure
PCO_2	Partial pressure of carbon dioxide
PEEP	Positive-end expiratory pressure
P_L	Transpulmonary pressure
SD	Standard deviation
SEM	Standard error of the mean
TK	Tachykinin
Vrx	Relaxation gas volume
Vrx'	Relaxation gas volume, mls per gram dry lung weight
W/D	Wet-to-dry

CHAPTER 1

PRELIMINARY EXPERIMENTS

INTRODUCTION

This study began as an investigation of the effects on the pulmonary circulation of chemical mediators released in allergic asthma, but turned instead to an examination of the phenomenon of massive postmortem bronchoconstriction in the guinea pig and hypocapnia-induced bronchoconstriction. While the initial studies of allergic asthma are not central to this thesis, they will be briefly described since they explain the events that led to a change in research direction.

Abnormalities of pulmonary gas exchange are frequent in asthma. The major cause is a mismatching of ventilation and blood flow within the lung. While airway obstruction is the most obvious factor involved (and the most frequently studied), both regional and total pulmonary blood flow changes are also thought to be important. Since there existed major deficiencies in knowledge of the behaviour of the pulmonary circulation in asthma, a detailed study of the pulmonary vascular response was proposed.

The specific object of these studies was to examine the effects on the pulmonary circulation of the various chemical mediators released in allergic asthma. This required an experimental model which would allow other variables, known to affect pulmonary blood flow, to be

controlled. An isolated lung preparation was chosen for the following reasons.

1) An isolated, perfused lung preparation makes it possible to avoid the confounding effects of gas trapping and alveolar overdistension. These two phenomena are common in asthma and can cause alterations in pulmonary blood flow in their own right. It was proposed not to ventilate the lungs during the experiment but instead, immediately after allergen aerosol challenge, to maintain lungs at a constant transpulmonary pressure.

2) It is possible with an isolated lung to exclude the effects of hypoxic pulmonary vasoconstriction. It was planned to perfuse the lungs with blood tonometered with a 5% CO_2: 30% O_2: 65% N_2 gas mixture and to use 5% CO_2: 95% O_2 to distend the lung.

3) The isolated lung is denervated, thus the effects of any neurogenic reflexes on blood flow can be avoided.

In order to do these studies it was necessary to a) produce a guinea pig model of allergic asthma and b) establish the methods for making measurements of the pulmonary vascular response in an isolated perfused lung preparation. These preliminary studies are described below.

METHODS

ALLERGIC MODEL

The model of Andersson (1980) was used because of its immunological similarity to human asthma. Preliminary **in vivo** experiments were conducted to confirm that the Andersson model of antigen-induced bronchoconstriction could be successfully reproduced. Eight adult albino guinea pigs (583 ± 116 gm, mean ± SD) were sensitized to ovalbumin by a single intraperitoneal injection of 1 *ug* ovalbumin (Sigma, Grade V), 100 mg dried aluminium hyroxide gel (Royal Adelaide Hospital Pharmacy) dissolved in 0.5 mls normal saline. Three weeks following sensitization seven animals (one died during the housing period) were anaesthetized by intraperitoneal pentobarbitone sodium (Nembutal® 30 mg/kg). The trachea was cannulated and connected to a small animal ventilator (Harvard model 665). The animals were ventilated with room air (40 cycles/min, tidal volume 4 mls, positive end-expiratory pressure (PEEP) 2 cm H_2O). Airway pressure was continuously monitored by a side-port connection from the tracheal cannula to a pressure transducer (Statham P230c) and recorded on a 4 channel polygraph (Grass Instruments). At this point three of the animals underwent bilateral vagotomy, thus giving two experimental groups of vagotomized (n=3) and non-vagotomized (n=4) animals. The tissue overlying the sternum was anaesthetized intradermally with 0.5% lignocaine. The chest was opened and the heart and lungs exposed. The intensity of bronchoconstriction was monitored by

measuring changes in dynamic compliance. Dynamic compliance (Cdyn) was calculated by dividing tidal volume by the difference between end-inspiratory and end-expiratory pressure at the airway opening. [Under open chest conditions, airway pressure at the airway opening is equal to transpulmonary pressure (P_L).] Each animal was first subjected to a fifteen second aerosol challenge of normal saline after which airway function was monitored for ten minutes. This was followed by an aerosol antigen challenge (15 seconds, 0.5% ovalbumin/normal saline solution) and again airway function was monitored for a further ten minutes. The aerosols were generated by a Turret nebulizer powered by an oxygen flow rate of 6 L/min. Aerosols were delivered to the airways via the ventilator.

ISOLATED LUNG PREPARATION

Initial protocol

Adult albino guinea pigs of either sex were anaesthetized with pentobarbitone sodium (60mg/kg). The tissue overlying the sternum was anaesthetized intradermally with 0.5% lignocaine. The animals were then tracheotomized and ventilated by a fixed volume cycled Harvard small animal ventilator (tidal volume 8 mls/kg, 40 breaths/min, PEEP 2 cm H_2O). The chest was opened under positive pressure ventilation. Heparin (1000 U/kg) was administered by an intracardiac injection (injection volume 1 ml/kg, 23G needle). The animals were quickly exsanguinated by left ventricular puncture collecting 20-30 mls blood

for the perfusate. Ventilation was ceased and the heart and lungs excised en bloc and then suspended by the tracheal cannula in a perspex chamber. Glass cannulae were inserted into the pulmonary artery, via the right ventricle, and into the left atrium, via the left ventricle. The cannulae were secured by a silk ligature around the atrio-ventricular groove. Great care was taken to avoid introducing air emboli into the pulmonary artery. The perspex chamber was then place on an electronic balance (Sauter K1200), enabling changes in lung weight to be constantly monitored.

The lungs were perfused (30 mls/min) with a mixture of 20 mls autologous blood and 30 mls of 4% bovine serum albumin (Boehringer Mannheim, Fraction V), Krebs-Henseleit solution (NaCl 118, KCl 4.75, CaCl 2.54, $KHPO_4$ 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25, glucose 5, mmol/L, pH balanced 7.40-7.45) using a roller pump (Masterflex, Model 7521-20). The resulting haematocrits were between 20-25%. Dilution of autologous blood in this way was necessary to obtain sufficient perfusate for the circuit. Pulmonary artery pressure was measured from a side hole catheter in the pulmonary artery cannula using a Statham pressure transducer (Model P231D). The perfusate temperature was maintained at 37°C by passing it through a glass heat-exchange coil which was placed in a heated water bath. The perfusate reservoir was kept in the same bath.

Perfusion was commenced within 10 minutes of stopping the animal's circulation, at which time ventilation was recommenced with the same

ventilator settings, using a 5% CO_2: 21% O_2: 74% N_2 gas mixture. Airway pressure, pulmonary artery pressure and lung weight were continuously recorded on a polygraph (Neotrace 600 ZEF). The isolated ventilation-perfusion lung system is shown in Figure 1.

Modified protocol

Because of recurrent postmortem bronchoconstriction (see results), it was decided to modify the experimental protocol to excluded exsanguination as a possible cause and to maintain pulmonary circulation during the isolation procedure. These modifications resulted from recent reports in the literature which implicated exsanguination as an initiator of postmortem bronchoconstriction in the guinea pig (Lai *et al*, 1984a, Lai *et al*, 1984b). Instead of exsanguinating the animal autologous blood was obtained by withdrawing small aliquots of blood (6-8 mls) from a peripheral vein followed by the immediate infusion of the same volume of Krebs-Henseleit solution. This exchange transfusion procedure produced 25-30 mls of venesected blood and final haematocrit values in the perfusate of 30-35%. To minimize interruption of the pulmonary circulation the pulmonary artery was cannulated **in situ** with a catheter being perfused (10-15 mls/min) with the Krebs-Henseleit solution. Artificial ventilation was not stopped. The lungs were then excised and transferred to the perspex chamber where the left atrium was cannulated. Perfusion rate was increased to 30 mls/min and the lungs were ventilated using a 5% CO_2: 21% O_2: 74% N_2 gas mixture.

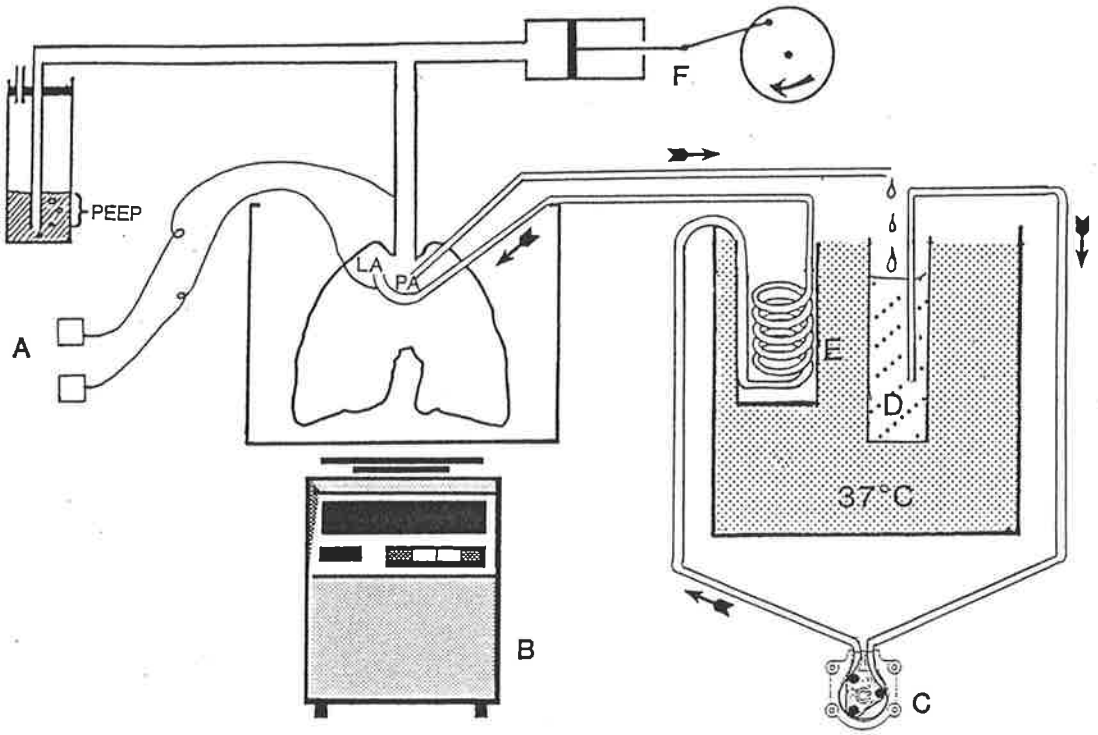


Figure 1. Schematic representation of the isolated, perfused-ventilated guinea pig lung preparation. Arrows indicate perfusion direction. Components of the circuit are

A Pressure transducers

B Electronic balance

C Roller pump

D Perfusate reservoir

E Heat exchanger

F Harvard ventilator

RESULTS

ALLERGIC MODEL

Changes in Cdyn with continuous ventilation following saline and antigen challenge are shown in Figure 2. Dynamic compliance did not change after saline aerosol but fell rapidly following antigen challenge, reaching a maximal response within 3 minutes which was maintained throughout the monitored period. Bilateral vagotomy appeared to make no difference to the changes in Cdyn that occurred following saline and antigen challenges.

ISOLATED LUNG PREPARATION

Initial protocol

The initial method of lung isolation yielded preparations in which postmortem lung function was quite unstable. Invariably the isolated lung preparation developed marked bronchoconstriction and gas trapping within 3-5 minutes of commencing mechanical ventilation, as evidenced by increased insufflation pressures (Figure 3) and lung overdistension (Figure 4).

Modified protocol

Seven guinea pigs were studied using this protocol. Despite lung

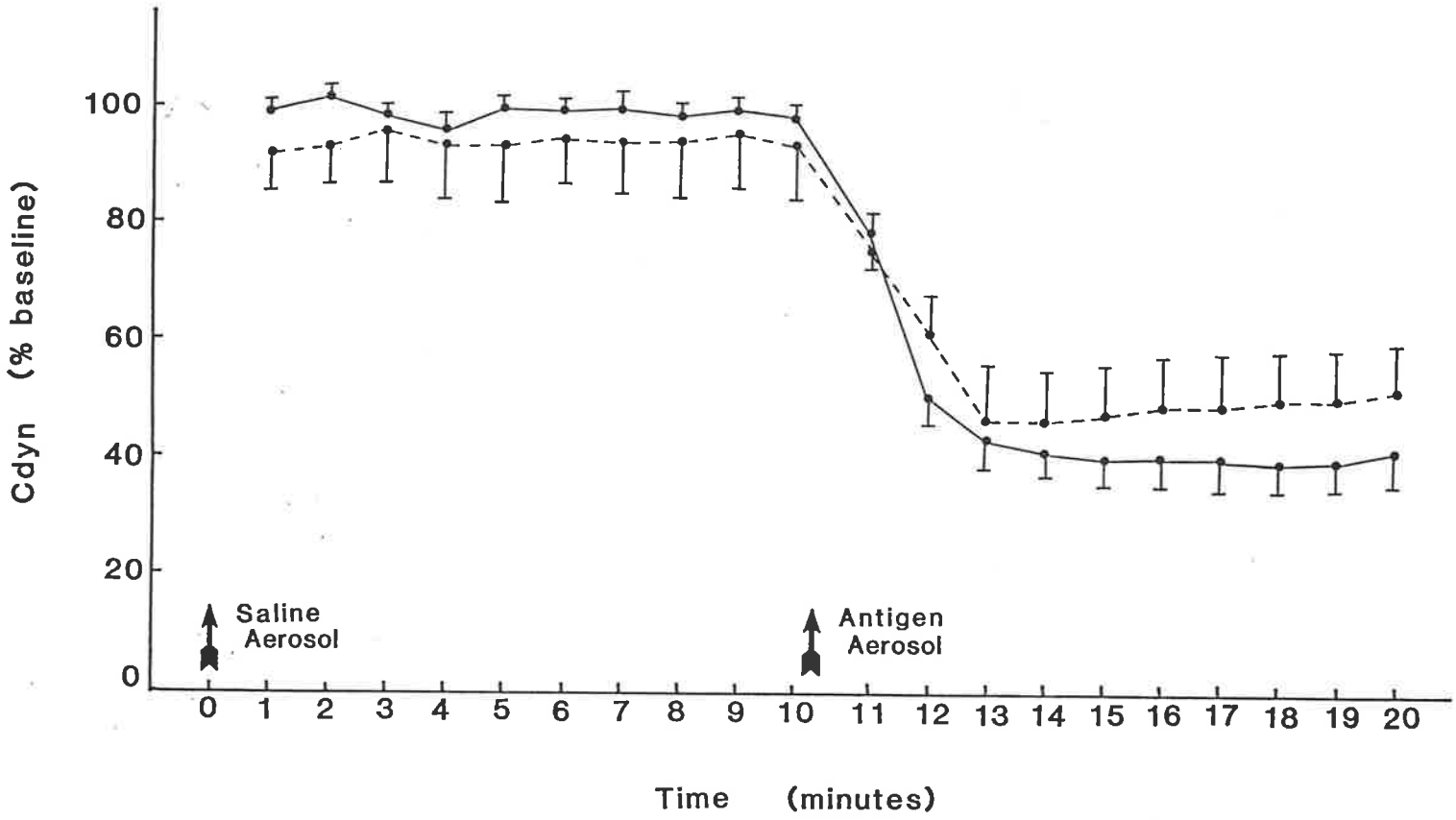


Figure 2. Changes in dynamic compliance (Cdyn) at one minute intervals following saline and antigen aerosol challenges in sensitized guinea pigs. Results are mean ± SEM for each group, ●——● vagus intact, ●--●vagotomized animals.

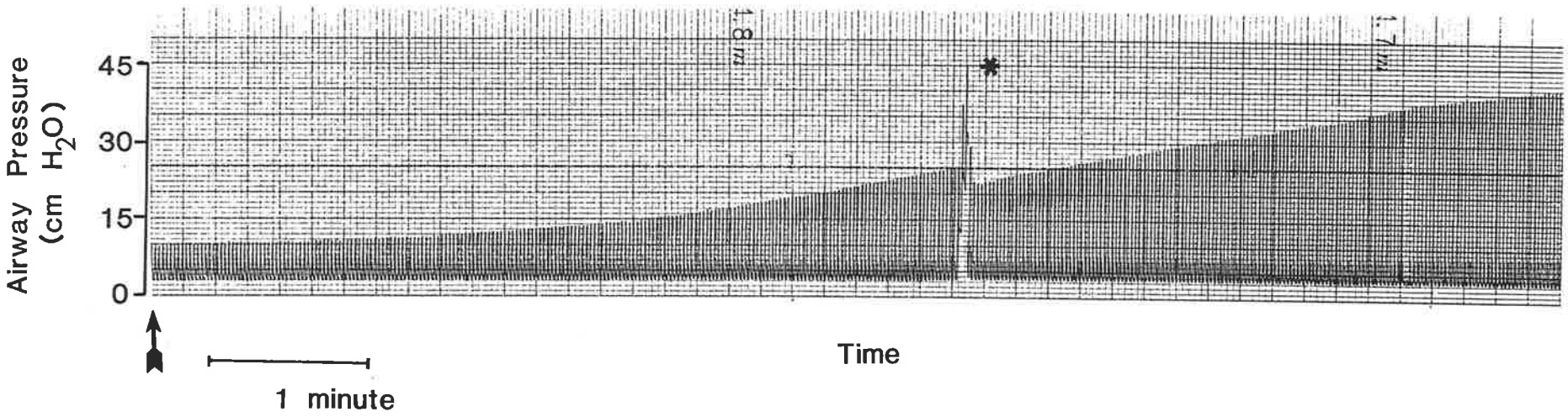
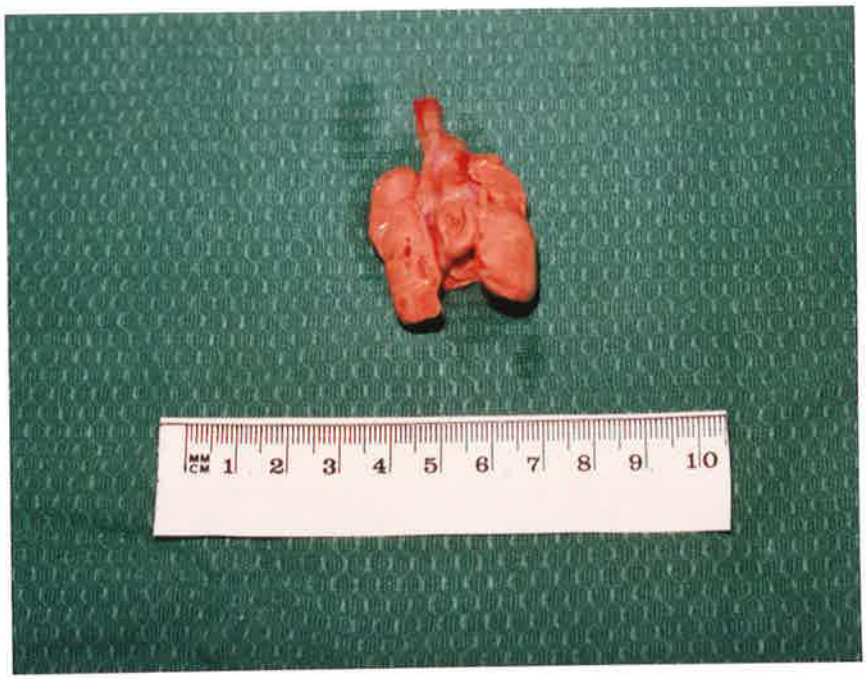


Figure 3. Changes in insufflation pressure with continuous ventilation of the isolated, perfused-ventilated guinea pig lung. Arrow indicates commencement of mechanical ventilation.

* Preparation was sighed to prevent atelectasis.

A



B

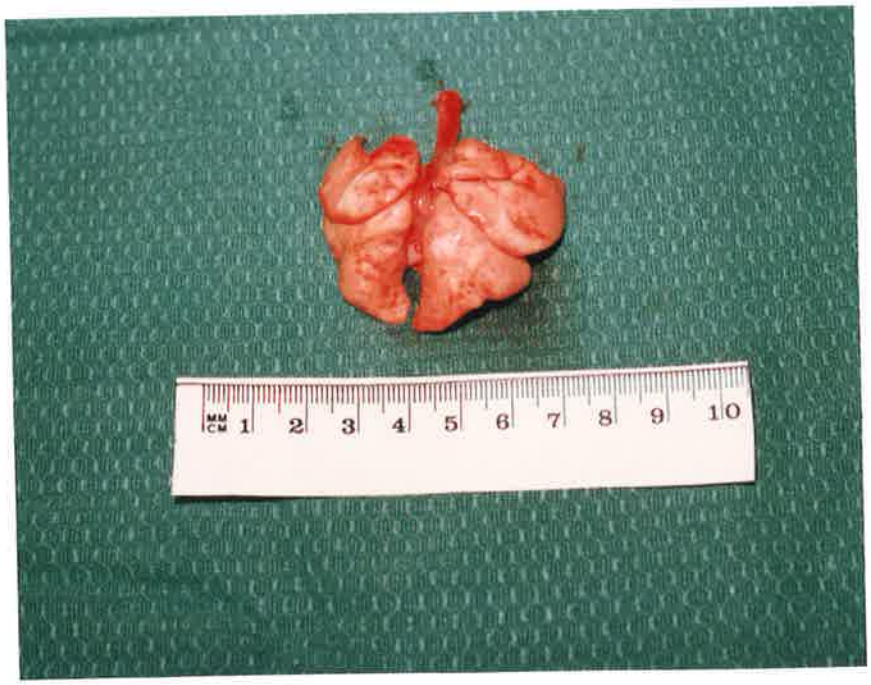


Figure 4. Photographs of excised guinea pig lungs.

A Non-ventilated lung.

B Isolated and ventilated lung.

perfusion during excision, bronchoconstriction still occurred, as evidenced by marked gas trapping and increased peak inspiratory airway pressure as seen with the initial protocol.

DISCUSSION

The capacity to reproduce the Andersson model of allergic asthma was confirmed by the **in vivo** studies. The results clearly demonstated the specificity of the airway response to ovalbumin, as no response occurred with saline challenge, and that this response was not vagally mediated. The latter finding was important since in some models of allergic asthma, e.g. Ascaris suum-sensitive dogs (Gold *et al*, 1972), the airway response is totally abolished by vagal blockade. In the present model, however, it was possible to conclude that there was sufficient release of chemical mediators within the lung following aerosol allergen challenge to produce bronchial smooth muscle contraction. It was the effects of these mediators on pulmonary vascular smooth muscle that was to be examined.

While it was confirmed by these preliminary experiments that allergen challenge released chemical mediators within airway walls, the occurrence of massive gas trapping in the isolated lung, even in the absence of aerosol challenge, effectively prevented a proper investigation of the effects of these mediators on the pulmonary circulation.

On reviewing the literature it was found that previous researchers in 1984 (Lai *et al*, 1984b) had shown the guinea pig to be susceptible to massive postmortem bronchoconstriction and these investigators had concluded that the mechanism involved a depletion in pulmonary blood volume caused by exsanguination. Lai and associates (1984a, 1984b) postulated that exsanguination, by unknown mechanisms, led to the release of substance P and in turn other bronchoconstrictors (Figure 5). Since the animals used in the initial protocol had been exsanguinated, it seemed possible that similar mechanisms might have been operating in the present experiments to induced bronchoconstriction.

Modifications of the initial protocol were therefore made in an attempt to maintain pulmonary blood volume and cause minimum disruption to the pulmonary circulation. Despite these modifications, massive bronchoconstriction still occurred. This led to a reconsideration of other factors known to induce bronchoconstriction e.g. hypocapnia (Cutillo *et al*, 1974), airway cooling and drying (Strauss *et al*, 1978).

A close review of the protocols (initial and modified) used during the lung isolation procedure in these preliminary experiments indicated that there may have been periods of relative hypocapnia. For example, in the initial protocol, mechanical ventilation was continued during exsanguination. This would have produced a brief period of hypocapnia. (It was also possible that ventilation during

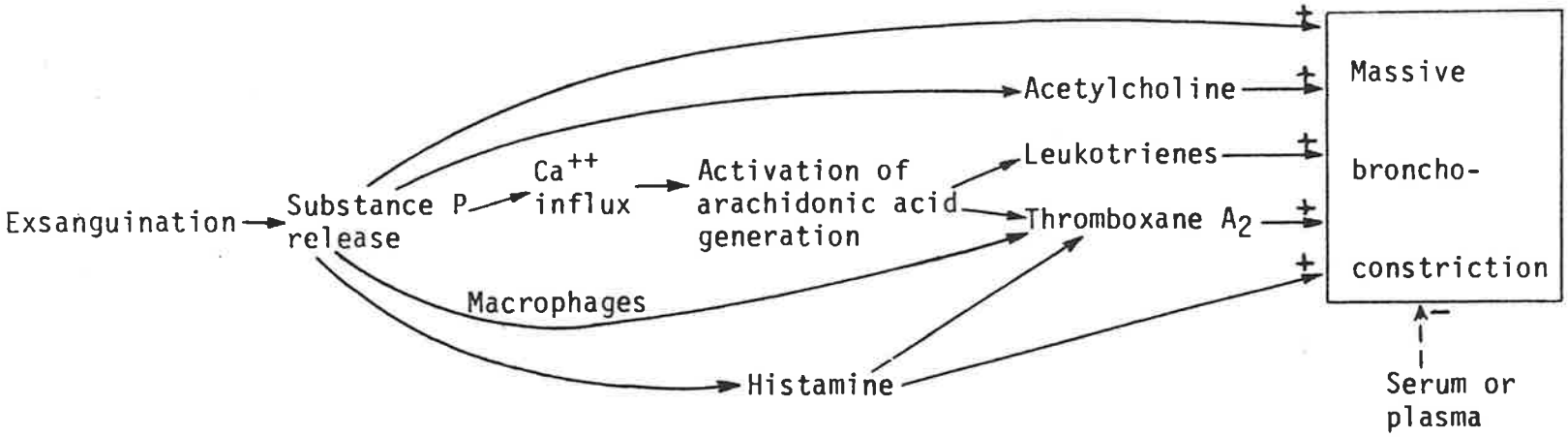


Figure 5. Proposed sequences of chemical mediator inducing or inhibiting massive bronchoconstriction following exsanguination. +, enhancement; -, inhibition. From Lai et al (1984a).

exsanguination could have led to some airway cooling and drying.) This period of hypocapnia could have been further extended when mechanical ventilation was reinstituted in the excised lung, since there would have been a delay in transit of the 5% CO_2 gas mixture through the ventilator and associated tubing. A similar problem was likely to have occurred in the experiments using the modified protocol. While lung temperature would have been maintained by the heated perfusate, the partial pressure of carbon dioxide (PCO_2) within the lung would have been minimal following the commencement of artificial perfusion. At this time delivery of CO_2 to the lung by venous blood had ceased. Since air ventilation was continued for several minutes and the perfusate had not been pre-tonometered with a CO_2 gas mixture the concentration of CO_2 within the airway lumen would have fallen. On conversion to a 5% CO_2 ventilating gas mixture alveolar and vascular compartments would have equilibrated within several recirculations, however, during the initial minutes of artificial perfusion the airways would have been exposed to hypocapnia.

These considerations led to a reappraisal of the factors causing massive postmortem bronchoconstriction in the guinea pig lung. Exsanguination, as suggested by Lai and associates (1984b), may have been a factor. However, it appeared from their methodology that they had not controlled the composition of airway gas in their experiments, and as in the present experiments, airway hypocapnia was likely to have occurred. Therefore, more carefully controlled

experiments were designed to systematically explore the relative importance of airway carbon dioxide concentration, humidity and temperature in postmortem lung function in the guinea pig. The results of these experiments are reported in the following chapter.

CHAPTER 2

MECHANISMS OF POSTMORTEM BRONCHOCONSTRICTION IN THE GUINEA PIG

INTRODUCTION

Isolated perfused lung preparations have become very important models in investigating various aspects of normal and altered lung physiology. The guinea pig isolated perfused lung is one such model that had been extensively used over past decades. Initially, it was intended to use this model in studies of the pulmonary circulation, but it was found that the preparation developed severe postmortem bronchoconstriction. A similar observation had been made previously by Lai *et al* (1984b), who conducted a series of experiments in an attempt to elucidate the mechanism (Lai *et al*, 1984a, Lai *et al*, 1984b). They found that severe bronchoconstriction developed within 10 minutes of exsanguination, whereas airway function remained normal for up to an hour if death was caused by 100% N_2 breathing (Lai *et al*, 1984b). From this it was concluded that the mechanism involved a depletion of pulmonary blood volume. As mentioned in the previous chapter preliminary experiments raised doubts about this conclusion and suggested that factors such as airway hypocapnia, airway drying and/or cooling were perhaps of equal or greater importance.

This chapter describes the experiments which were designed to systematically test the effects of varying airway gas composition on postmortem lung function, in the absence of a change in pulmonary blood volume. The results show that hypocapnia, in particular, is a potent stimulus for bronchoconstriction in the postmortem guinea pig lung.

METHODS

Hartley strain guinea pigs (Waite Agricultural Institute - Adelaide) weighing 580 ± 130 gm (mean ± SD) were anaesthetized by intraperitoneal pentobarbitone sodium (60 mg/kg) and the tissue overlying the sternum was anaesthetized intradermally with 0.5% lignocaine. The trachea was cannulated and connected to a small animal ventilator (Harvard model 665). The animals were ventilated with room air (40 cycles/min, tidal volume 8 mls/kg, PEEP 2 cm H_2O). Airway pressure was continuously monitored by a side port connection from the tracheal cannula to a pressure transducer (Statham P230c) and recorded on a polygraph (Neotrace 600 ZEF). At this point all animals underwent bilateral vagotomy. The chest was opened and the heart and lungs exposed, and a silk ligature placed around the heart in the atrio-ventricular groove. Animals were sacrificed by quickly tightening, then tying the heart ligature. This produced sudden circulatory arrest while maintaining the pulmonary blood volume at a level similar to that existing immediately antemortem (right ventricular outflow and left ventricular filling were stopped virtually simultaneously). Following cardiac ligation animals were divided into five groups.

Control animals (n=6); immediately following heart ligation ventilation was ceased and the lungs were excised to provide control data on the volume of trapped gas and wet-to-dry (W/D) weight ratios

(see below). The remaining animals were divided into 4 groups according to the type of gas used for postmortem **in situ** lung ventilation. Group 1 (n=10); room air. Group 2 (n=10); conditioned air. Group 3 (n=10); a dry 5% CO_2; 21% O_2; 74% N_2 gas mixture at room temperature. Group 4 (n=10); a conditioned 5% CO_2; 21% O_2; 74% N_2 gas mixture. For Groups 2 and 4 the inspired gas was conditioned by bubbling it through a series of heated (45°C) water chambers. Temperature at the airway opening was measured with an in-line electronic thermistor (Yellow Springs 400). The water content of inspired gas was determined before the experiments by directing a large gas sample, of known volume, from the ventilator through a series of anhydrous calcium sulphate tubes. Humidity was then calculated from the change in weight of the drying tubes and the sampled gas volume. Temperature and humidity were 37°C and 91% and 34°C and 83% for Groups 2 and 4 respectively.

Dynamic compliance (Cdyn), as mentioned previously, was calculated by dividing tidal volume by the transpulmonary pressure difference at points of zero flow i.e. end-inspiration and end-expiration. Since a change in Cdyn may result from a number of events occurring within the lung (see discussion), it may be described as a non-specific indicator of abnormality in lung function. A decrease in Cdyn may result from 1) airway narrowing (e.g. smooth muscle contraction), 2) a decrease in lung distensibility because of a change in the lung's elastic properties (e.g. pulmonary oedema) or 3) the closure of some lung units (e.g. basal atelectasis). One would expect to observe, in

the latter two cases, a decrease in lung trapped gas volume while the former to result in an increase. In order to distinguish between the above possibilities relaxation volume was measured (vide infra) in addition to Cdyn.

In Groups 1, 2, 3 and 4 ventilation was ceased 15 minutes after cardiac ligation, PEEP was removed and the lungs allowed to deflate to a resting P_L of zero. In Control animals ventilation was stopped simultaneously with cardiac ligation and the lungs also allowed to passively deflate. The trachea was then ligated with a silk suture and the lungs excised, dissected free of heart, oesophagus, and connective tissue, and weighed. Lung volume at P_L=0 was determined by direct saline displacement (Drazen and Austen, 1975). The volume of gas within the lung was calculated by subtracting the volume of lung tissue from the displacement volume. (Tissue volume was calculated from wet lung weight using an assumed tissue density of 1.06 gm/ml (Wohl *et al*, 1968).) The volume of gas at P_L=0 was termed relaxation volume (Vrx) and was used as an indicator of trapped gas. Relaxation volume was normalized for lung size by dividing Vrx by the dry lung weight to give a corrected volume Vrx'. Dry lung weight was obtained by placing the lungs in a drying oven until their weights on daily determinations no longer decreased. Lung wet-to-dry (W/D) weight ratios were calculated.

Statistics

An analysis of variance for randomized block design was used to test separately Groups 1, 2, 3 and 4 for significant changes in Cdyn over time. One way analysis of variance and the Bonferroni test (Wallenstein *et al*, 1980) were used to determine whether significant differences existed between group mean values. $P < 0.05$ was considered to be significant.

RESULTS

Changes in Cdyn with continuous ventilation following cardiac ligation are shown in Figure 6. Animals ventilated with room air (Group 1) had a marked fall in Cdyn from baseline (mean $\pm$ SEM, 50 $\pm$ 5% at 15 minutes). The onset of the response was rapid with a statistically significant decrease in Cdyn at 5 minutes which was maintained for the next 10 minutes. There was no significant change when the room gas was conditioned (Group 2), but when 5% CO_2 was added to the inspirate and the gas was conditioned (Group 4) there was a marked blunting of the postmortem airway response. Postmortem ventilation with a dry, room temperature 5% CO_2 gas (Group 3) produced postmortem changes in Cdyn that were intermediate between Group 1 and Group 4 responses, indicating that airway drying and/or cooling can also lead to alterations in postmortem airway function in this model if sufficient stimulus is applied.

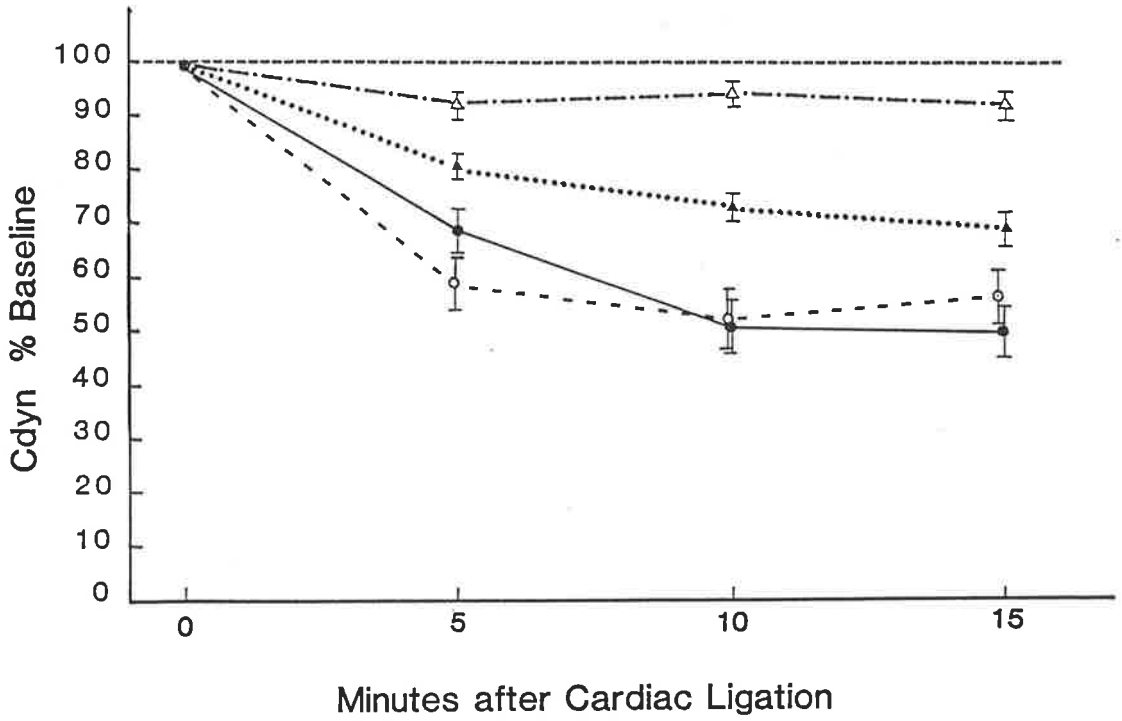


Figure 6. Changes in dynamic compliance (Cdyn) with continous ventilation following cardiac ligation. Given values are mean ± SEM. O-O: room air ventilation, ●—●: conditioned air, ▲··▲: dry, room temperature 5% CO_2; 21% O_2; 74% N_2, Δ··Δ: conditioned 5% CO_2; 21% O_2; 74% N_2. There was a significant decrease in Cdyn over time in all groups. There was no significant difference between Group 1 and 2 values at 5, 10 and 15 mins. There were significant differences at 5, 10 and 15 mins between mean values of Group 1 and 3, Groups 1 and 4, and Groups 3 and 4.

Figure 7 summarizes the Vrx per gram dry lung weight data for each experimental group. Group 1 and Group 2 animals had significantly more trapped gas than Controls. The relaxation gas volumes in Groups 3 and 4 were not significantly different from Controls. Wet-to-dry weight ratios are given in Table 1. None of the mean W/D weight ratios for the experimental groups was significantly different from Control values, although the value for Group 3 animals was lower than all others and approached statistical signficance ($p<0.10$), suggesting that ventilation with dry gas in this group produced some airway drying.

DISCUSSION

It was found that postmortem ventilation of guinea pig lungs with room air resulted in a rapid decrease in Cdyn and marked gas trapping. Similar changes were noted previously by Lai and colleagues (1984b) who also showed histologic evidence of intense bronchial smooth muscle constriction. They considered the most likely cause was a reduction in pulmonary blood volume during exsanguination. This seemed an unlikely explanation, however, for the present results since animals were sacrificed by sudden cardiac ligation, a technique designed to maintain postmortem pulmonary blood volume at antemortem levels.

There are several possible explanations for the postmortem decrease in Cdyn observed in these experiments: 1) airway narrowing due to

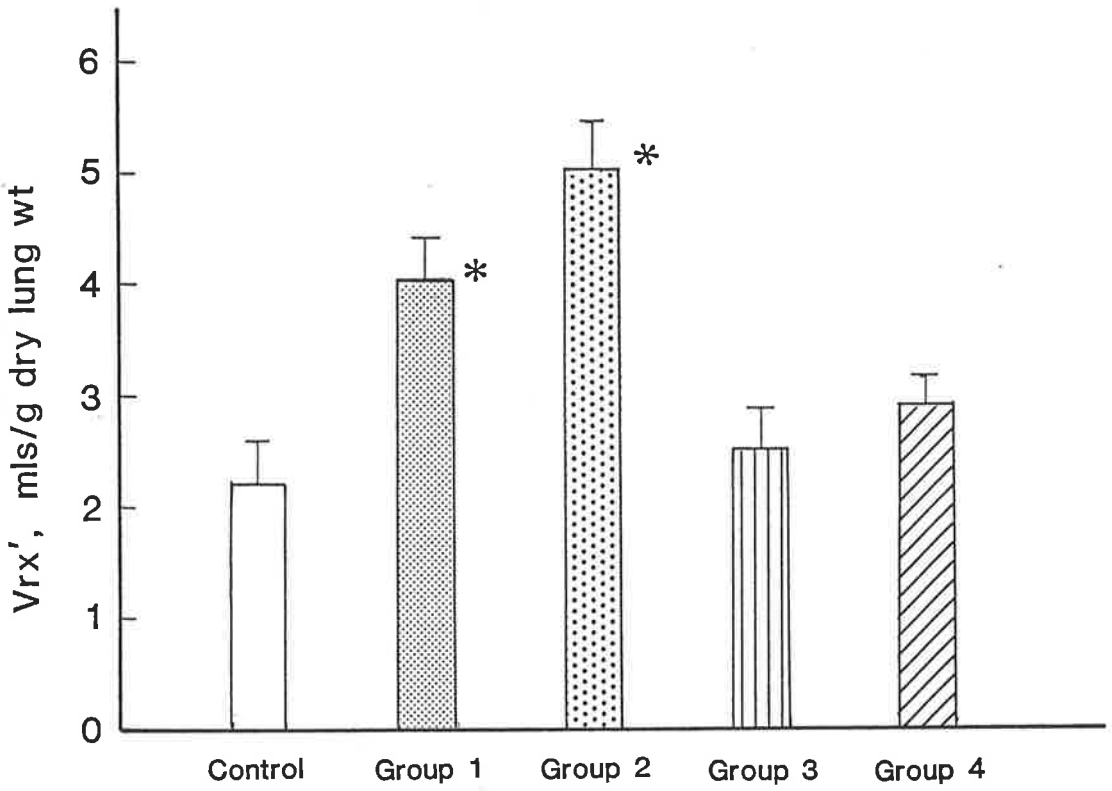


Figure 7. Relaxation volume in mls per gram dry lung weight (Vrx') for control and experimental groups. Data are means ± SEM. Group 1 lungs were ventilated with room air, Group 2 with conditioned air, Group 3 with dry, room temperature 5% CO_2; 21% O_2; 74% N_2, and Group 4 with conditioned 5% CO_2; 21% O_2; 74% N_2. * Significant compared with Control group, $P<0.05$.

TABLE 1. *Wet-to-dry weight ratios for guinea pig lungs*

	Group				
	Control	*1*	*2*	*3*	*4*
n	6	10	10	10	10
Wet-to-dry weight ratio	5.09±0.03	4.88±0.10	5.06±0.22	4.58±0.11	5.22±0.11
Significant values		NS	NS	NS	NS

Values are means ± SE. Significant values are compared with control values.

smooth muscle constriction, peribronchial oedema or mucus production, 2) a decrease in lung distensibility because of a depletion or change in the physical properties of surfactant, or because of oedema formation, and 3) airway closure and a loss of functional lung units.

An 88% increase in gas trapping, was observed, after 15 minutes of room air ventilation which indicated that the reduction in Cdyn was at least in part due to airway narrowing and airway closure. It was clear that this airway narrowing could not have been the result of reflex bronchoconstriction (e.g. stimulation of chemoreceptors by hypercapnia (Nadel and Widdicombe, 1962)) as all animals underwent bilateral vagotomy prior to the experiments. It also seemed unlikely that it was the result of peribronchial oedema. The lungs did not become oedematous postmortem as judged by W/D weight ratios, and bronchial wall oedema would therefore have required a rapid shift of fluid from some other part of the lung (e.g. alveolar walls). This seemed improbable as intravascular pressures would rapidly equilibrate in the lung after circulatory arrest. Intraluminal oedema fluid is an unlikely explanation for similar reasons. An accumulation of intraluminal mucus also seemed unlikely, given the very rapid onset of the airway reaction (less than 5 minutes) and the previous vagotomies. Although, as decribed in Chapter 3, release of tachykinins via a local bronchial axonal reflex could in theory stimulate mucous gland secretion.

The results of further experiments suggested that postmortem airway

hypocapnia was primarily responsible for the airway narrowing and gas trapping. During postmortem ventilation with room air both airway hypocapnia and airway cooling and drying would have occurred, and both factors are known to cause bronchoconstriction. The fact that in these experiments simply conditioning room air gas did not alter the abnormal postmortem pulmonary mechanics, whereas the addition of 5% CO_2 produced marked improvements, suggested that airway hypocapnia was the main factor involved. Previous studies have shown that a reduction in airway carbon dioxide partial pressure ($P_{aw}CO_2$) can produce marked peripheral airway constriction in both human and animal lungs (Cutillo *et al*, 1974, Ingram, 1975, Newhouse *et al*, 1964, Severinghaus *et al*, 1961, Sterling, 1968, Swenson *et al*, 1961). It is clear that in the first experimental group the continued ventilation of lungs after circulatory arrest with room air at physiologic frequency and tidal volume would have reduced $P_{aw}CO_2$ to negligible levels within a few minutes. Severinghaus and colleagues (1961) showed in dogs that a reduction in $P_{aw}CO_2$ to approximately 10 Torr was sufficient to produce bronchial smooth muscle constriction.

While these data suggest a predominant role for airway hypocapnia in postmortem bronchoconstriction, experimental results obtained using a dry, room temperature 5% CO_2 gas mixture indicated that the postmortem guinea pig lung may also be adversely affected by airway cooling and drying. During postmortem ventilation with this gas mixture, gas trapping did not occur but a significant decrease in Cdyn was apparent. These changes could have been due to

brochoconstriction (without airway closure) and/or a decrease in lung distensibility. Both phenomena may have occurred and could be explained by the effects of lung cooling and drying.

Significant lung cooling and dehydration almost certainly occurred during ventilation with the dry, room temperature CO_2-enriched gas. Faridy, Permutt and Riley (1966) showed that the temperature of excised, non-ventilated dog lobes equilibrated with the environmental temperature in approximately 10 minutes. It seemed likely that lung cooling would have been even more rapid in this open-chest preparation because ventilation was continued postmortem with the room temperature gas. The observation that W/D weight ratios of Group 3 lungs were reduced suggested that some airway dehydration also occurred during ventilation with the unconditioned gas. This would also have contributed to airway surface cooling through evaporative heat loss.

Airway cooling can lead to bronchoconstriction in humans (Deal *et al*, 1979, O'Cain *et al*, 1980) and increased smooth muscle histamine reactivity in experimental animals (Souhrada and Souhrada, 1981). Dehydration of the surface airway lining may also have had a bronchoconstrictor effect (Hahn *et al*, 1984). It is possible, therefore, that in the present study these two factors (airway cooling and drying) led to some postmortem bronchoconstriction and were responsible for the decrease in Cdyn observed during ventilation with the unconditioned 5% CO_2 gas mixture. An alternative

explanation is that lung distensibility decreased due to a change in lung surfactant. Static lung compliance has been shown to decrease significantly in isolated rat lungs after 15 minutes continuous ventilation at room temperature (McClenahan and Urtnowski, 1967). Possible reasons are 1) a direct effect of lung cooling on the physicochemical properties of surfactant (Lempert and Macklem, 1971) or 2) a decrease in the capacity of alveolar type II cells to adequately replenish surfactant during mechanical ventilation (Faridy *et al*, 1971, McClenahan and Urtnowski, 1967).

The studies of Lai *et al* (1984a, 1984b) were reviewed, to determine whether postmortem $P_{aw}CO_2$ changes could possibly be invoked to explain some of the massive bronchoconstriction and gas trapping they observed in the same animal model. It was apparent from the descriptions of ventilatory manoeuvres carried out in their first series of experiments (Lai *et al*, 1984b), that lungs of guinea pigs used in those particular experiments would also have been exposed to marked reductions in $P_{aw}CO_2$. They conducted essentially two types of postmortem ventilatory maneuvre: 1) Continuous ventilation with gas mixtures deficient in CO_2 (100% O_2 and 100% N_2), and 2) two maximal inflation-deflation manoeuvres with room air. The former would have reduced $P_{aw}CO_2$ to zero, and the latter would have decreased $P_{aw}CO_2$ by a factor of approximately 10 to a level of about 4-5 Torr. Therefore, in both cases decreases in $P_{aw}CO_2$ would have alone been sufficient to cause the postmortem bronchoconstriction reported. It is more difficult to be certain about the role of $P_{aw}CO_2$ changes in the

postmortem bronchoconstriction observed in their subsequent study (Lai *et al*, 1984a). They found that lungs which were perfused with a CO_2-containing artificial perfusate still developed air trapping. However, room air ventilation was apparently continued while the pulmonary circulation was interrupted and the artificial perfusion commenced. Airway P_{CO2} would presumably have decreased during that brief interval but whether the fall would have been sufficient to trigger bronchoconstriction is unknown.

Lai and colleagues (1984b) concluded that pulmonary blood volume depletion was important in the genesis of postmortem airway constriction partly because non-exsanguinated animals sacrificed by 100% N_2 ventilation did not develop immediate postmortem bronchoconstriction. However, results obtained after this method of sacrifice may have been misleading since **in vitro** experiments have shown hypoxia to impede the contraction of airway smooth muscle caused by histamine (Nisell, 1950) and electrical excitation (Stephens *et al*, 1968). Moreover, Severinghaus *et al* (1961) showed in **in vivo** experiments that 100% N_2 ventilation prevented hypocapnia-induced airway smooth muscle constriction. Therefore, the absence of postmortem bronchoconstriction in their animals sacrificed by 100% N_2 breathing (Lai *et al*, 1984b) may have been due to a loss of airway smooth muscle tone rather than maintenance of pulmonary blood volume.

Lai and associates (1984a, 1984b) conducted experiments to examine

the role of various biochemical mediators in severe postmortem bronchoconstriction. They found that bronchoconstriction was markedly attenuated following the depletion of substance-P by capsaicin pretreatment and by prior administration of the thromboxane synthesis inhibitor, dazoxiben. These results, taken together with the present observations, raised the possibility that airway hypocapnia, and possibly airway drying and cooling, produced bronchoconstriction through the release of tachykinins and arachidonic acid metabolites.

It is possible that the bronchial smooth muscle response to changes in $P_{aw}CO_2$ is more intense in the guinea pig lung than other mammalian species. One possible explanation is the apparent increase in bronchial smooth muscle in the guinea pig compared with other species (McLaughlin *et al*, 1966). However, postmortem gas trapping with lung inflation and deflation has also been observed in the dog (Faridy *et al*, 1966), rat (Frazer and Weber, 1976), rabbit (Lempert and Macklem, 1971) and sheep (Lum and Mitzner, 1985). While some authors have attributed this to the formation of stable airway "bubbles" behind which gas is trapped (Frazer *et al*, 1979), evidence of foam production has been obtained in one study only (Faridy and Permutt, 1971). The present results have provided indirect evidence for marked postmortem bronchoconstriction in the guinea pig when $P_{aw}CO_2$ was reduced and Lai and associates (1984b) have shown direct histologic evidence of bronchial smooth muscle constriction in the same animal model under similar experimental conditions. It seemed possible

therefore that hypocapnia-induced bronchoconstriction may have been responsible, at least in part, for air trapping in some of these previous experiments (Frazer and Weber, 1976, Lempert and Macklem, 1971, Lum and Mitzner, 1985) in which $P_{aw}CO_2$ was not controlled.

In summary, it has been shown that guinea pig lungs developed marked gas trapping and reduced Cdyn when ventilated for 15 minutes postmortem with room air. These changes did not appear to depend on a decrease in pulmonary blood volume. Instead, they appeared to be primarily due to airway hypocapnia. This model can, however, also develop abnormal postmortem pulmonary mechanics in the presence of marked airway cooling and drying. It is of considerable importance to those experimenting with the isolated guinea pig lung to known that normal or near normal airway function can be preserved (at least in the short-term) if attention is given to maintaining normal physiologic properties of airway gas.

The most striking finding in these studies was the intense bronchoconstrictor response in the guinea pig to airway hypocapnia. Hypocapnia-induced bronchoconstriction (HIBC) has been recognized for over 3 decades but to now has not been explained. This model seemed ideally suited to a systematic study of this phenomenon. Of particular interest were the findings of Lai *et al* (1984a) in relation to substance P. As argued above it seemed likely that

massive postmortem bronchoconstriction in their experiments was principally due to airway hypocapnia. There was therefore, a strong possibility that HIBC was mediated by substance P and other related tachykinins. This hypothesis was tested in the experiments reported in the following chapter.

CHAPTER 3

THE ROLE OF TACHYKININS IN HYPOCAPNIA-INDUCED BRONCHOCONSTRICTION

INTRODUCTION

Airway hypocapnia causes bronchoconstriction in a number of different species, including man (Cutillo *et al*, 1974, Jamison *et al*, 1987, Kolbe *et al*, 1987, Newhouse *et al*, 1964, O'Cain *et al*, 1979, Severinghaus *et al*, 1961, Sterling, 1968). It is thought that this response may be beneficial in the presence of lung disease because it directs ventilation away from poorly perfused lung regions, thereby reducing ventilation-perfusion inequality. Despite the fact that hypocapnia-induced bronchoconstriction (HIBC) has been recognized for over 30 years, its mechanisms have to now remained essentially unknown.

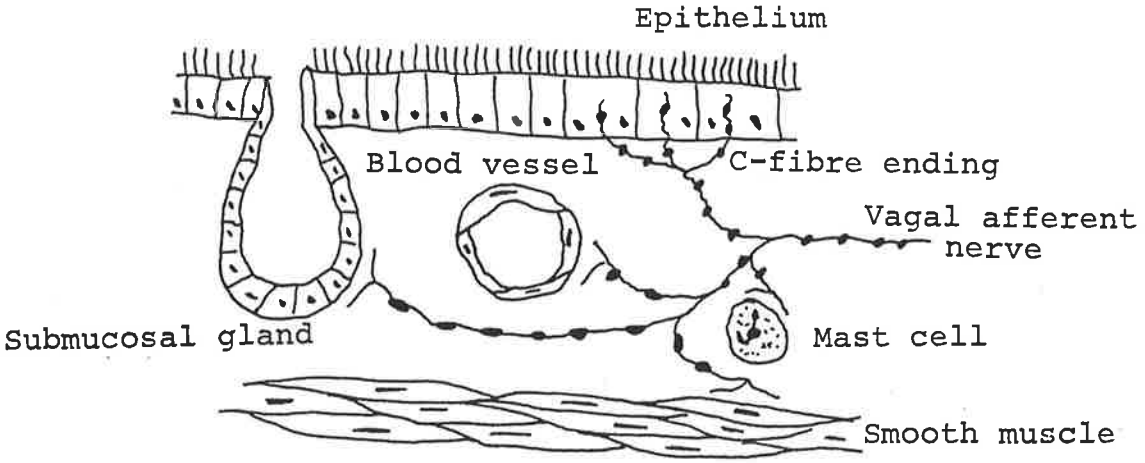
It was found in some human studies (Newhouse *et al*, 1964, Sterling, 1968) that HIBC was attenuated by atropine suggesting the involvement of a vagal cholinergic reflex. In other human studies anticholinergic drugs produced no effect (Jamison *et al*, 1987, O'Cain *et al*, 1979) and in dogs, HIBC was not affected by either vagotomy, atropine or ganglionic blockade (Severinghaus *et al*, 1961). Also, it appeared from recent experiments that neither histamine nor prostaglandins played a significant role in HIBC (Kolbe *et al*, 1987).

The results of experiments reported in Chapter 2 strongly implicated hypocapnia as the trigger for postmortem bronchoconstriction in the guinea pig. Several studies (see Lai *et al*, 1984a, Lai and Cornett, 1987) had implicated substance P in postmortem bronchoconstriction.

It seemed reasonable therefore to hypothesize that substance P and other tachykinins mediated HIBC. In this chapter experiments using the postmortem guinea pig lung model are described in which the role of tachykinins (TKs) in HIBC were investigated.

Tachykinins are a group of neuropeptides which share a similar amino acid sequence at their C-terminal end. For many years substance P was considered to be the only tachykinin present in mammals. However, in the last five years three new tachykinins were isolated i.e. neurokinin A (also called substance k, neurokinin α or neuromedin L) neurokinin B (also called neurokinin β or neuromedin K) and neuropeptide K. Tachykinins are found widely in the central and peripheral nervous systems. In the lung, they appear to co-localize in non-myelinated sensory (C-afferent) nerve fibres with another neuropeptide, calcitonin gene related peptide (Martling, 1987a). Tachykinins have a variety of biological effects including bronchial smooth muscle contraction (Martling *et al*, 1987b). They are released from non-myelinated sensory nerves, following activation of nerve terminals and antidromic sensory nerve conduction. This type of nerve transmission is known as an "axonal reflex". This concept is portrayed schematically in Figure 8. Because C-afferent nerve terminals have been found histologically within airway epithelium (Lundberg *et al*, 1984), it was questioned whether airway hypocapnia could induce bronchoconstriction by activating a bronchial axonal reflex to release TKs. To investigate this possibility three different experimental approaches were used. Firstly, the effects of

NORMAL



HYPOCAPNIA

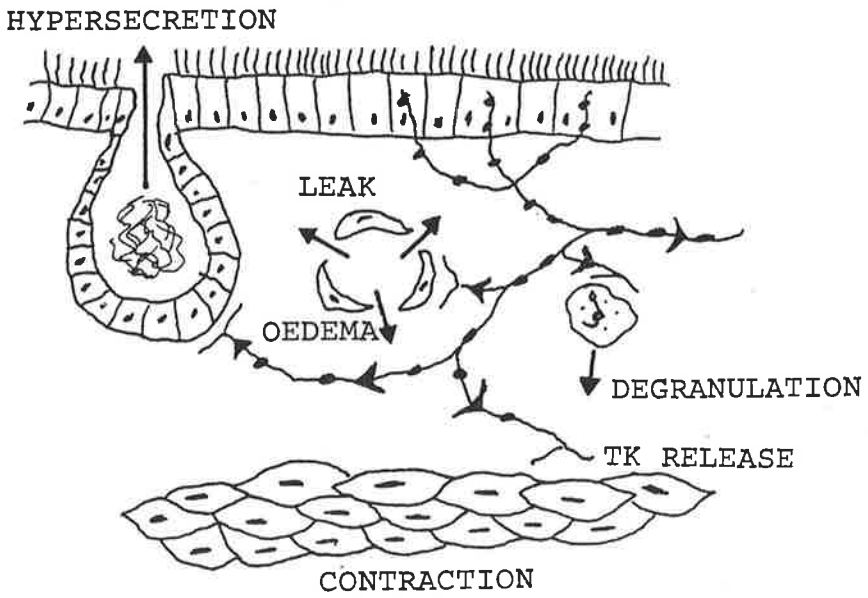


Figure 8. Axonal reflex mechanism. Unmyelinated C-fibre endings in the airway epithelium may be stimulated by airway hypocapnia, resulting in antidromic spread of impulse through terminal arborizations causing the release of tachykinins. These in turn may produce bronchoconstriction, mucus hypersecretion, microvascular leakage and mast cell degranulation. Modified from Barnes (1986).

TK depletion (by repeated capsaicin injections) on HIBC were investigated. Secondly, possible augmentation of HIBC by pretreating guinea pigs with an inhibitor of enkephalinase (EC 3.4.24.11 (Borson *et al*, 1987)), the main enzyme responsible for the rapid degradation of TKs was examined; and thirdly, topical anaesthesia was applied to the airways of vagotomized animals in an attempt to block activation of an axonal reflex.

METHODS

Experiments were performed on albino Hartley strain guinea pigs of both sexes. All animals used in the following series of experiments were prepared in the same manner, using methods similar to those described in the previous chapter. They were first anaesthetized by intraperitoneal pentobarbitone sodium (60 mg/kg). The trachea was cannulated and connected to a small animal ventilator (Harvard model 665) and ventilation commenced with room air (40 cycles/min, tidal volume 5 mls, positive end-expiratory pressure (PEEP) of 2 cm H_2O). Airway pressure was continuously monitored and recorded on a polygraph (Neotrace 600 ZEF) using a side-port catheter connected from the tracheal cannula to a pressure transducer (Statham P230c). All animals then underwent bilateral cervical vagotomies following which the chest was opened, the heart and lungs exposed, and a silk ligature placed in the atrio-ventricular groove. The animals were killed by quickly tightening then tying the heart ligature to produce sudden circulatory arrest. Following death the lungs were

continuously ventilated **in situ** for 15 minutes with gas of varying CO_2 concentration (see below), which was heated and humidified as described in the previous chapter.

HIBC following capsaicin pretreatment.

Eighteen animals (578 ± 102 gm, mean ± SD) were used in this study. Capsaicin (8-methyl-N-vanillyl-6-nonenamide, the irritant compound of chilli peppers) causes the release of TK stores from afferent nerve fibres. When injections are given repeatedly in adult guinea pigs, TK stores become depleted and there is degeneration of peripheral C-afferent nerve fibres which can last over a year (Buck *et al*, 1981, Papka *et al*, 1984). The protocol used in this study to deplete TK stores was a slight modification of that described by Papka *et al* (1984). Briefly, capsaicin (Sigma chemicals) was dissolved in a vehicle containing 3:1, ethanol : Tween 80 and given subcutaneously in the back of the neck over 5 successive days: Day 1, 2 mg capsaicin/kg body weight; Day 2, 20 mg/kg; Day 3, 100 mg/kg; Day 4, 200 mg/kg; Day 5, 400 mg/kg. The capsaicin solutions were constituted such that injection volume was the same on each day (1ml/kg). Following the injections of capsaicin on days 1 and 2 the animals (n=8) were immediately placed in a chamber premisted with an aerosol of salbutamol (0.5% w/v, Glaxo). This was not necessary for subsequent doses as animals became insensitive to the irritant effects of capsaicin. Control animals (n=10) were subjected to the same protocol of injections using the vehicle only. Seven days after

the last injection the animals were prepared as described above, sudden circulatory arrest was produced by cardiac ligation, and the lungs were ventilated with a heated (37°C), humidified (91%) hypocapnic gas mixture (0% CO_2: 21% O_2: 79% N_2).

As described in Chapter 2, dynamic compliance (Cdyn) was calculated by dividing tidal volume by the difference between end-inspiratory and end-expiratory pressure at the airway opening and at fifteen minutes after cardiac ligation ventilation was ceased and relaxation lung volume (Vrx) determined by saline displacement. Relaxation volume was subsequently normalized for lung size by dividing by the dry lung weight to give a corrected volume, Vrx'.

HIBC following enkephalinase inhibition.

A preliminary study was performed to produce a dose response curve for airway CO_2. Using the same animal preparation, the lungs of 30 guinea pigs (472 $\pm$ 127 gm, mean $\pm$ SD) were ventilated postmortem with heated (37°C) and humidified (95%) gas of different CO_2 compositions: 0%, 1.27%, 2.37%, 3.24%, 4.23% and 5.13% in 21%O_2, the balance being made up of N_2 (n=5, each group). The effects of increasing inspired CO_2 concentration on Cdyn and Vrx' are shown in Figures 9 and 10 respectively. From the observed dose response relationship the 2.37% CO_2 gas mixture was chosen for subsequent experiments because it produced an intermediate degree of bronchoconstriction. Experiments

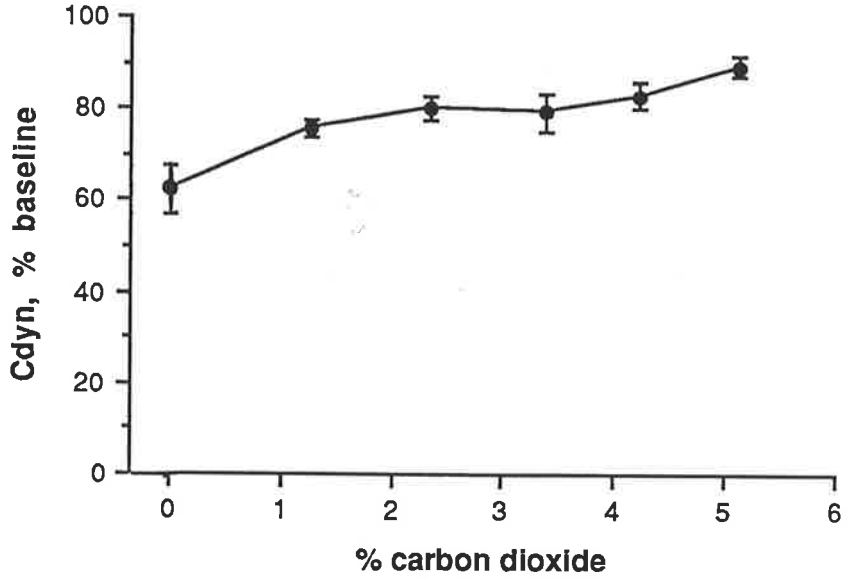


Figure 9. Dose-response relationship of airway CO_2 concentration and dynamic compliance (Cdyn) measured following 15 minutes ventilation post cardiac ligation. Values are expressed as percent of baseline, mean $\pm$ SEM.

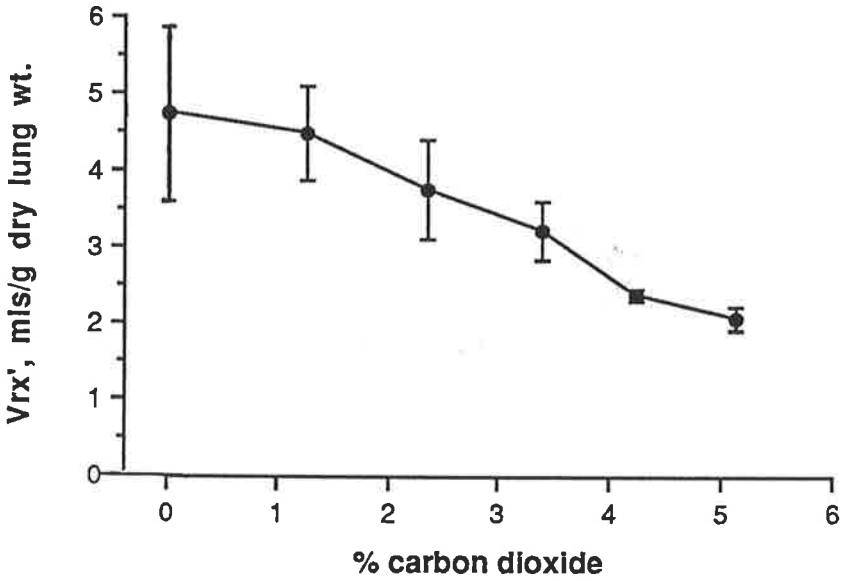


Figure 10. Dose-response relationship of airway CO_2 concentration and relaxation volume (Vrx') measured following 15 minutes ventilation post cardiac ligation. Values are expressed as means $\pm$ SEM.

were designed to test whether this sub-maximal response could be potentiated by pretreatment with the enkephalinase inhibitor, phosphoramidon.

For the enkephalinase inhibition studies the right internal jugular vein was cannulated and the cannula kept patent by filling its dead space with heparinized saline (1000 U/ml). Guinea pigs (490 ± 151 gm, mean ± SD) were divided into experimental and control groups (n=6, each group). In the experimental group, phosphoramidon (Sigma chemicals) was infused (10^{-5} moles/kg) over 1 min, 2 mins prior to cardiac ligation. In control animals the same volume of normal saline was infused. Following cardiac ligation the lungs were ventilated for 15 minutes with heated (37°C) and humidified (95%) gas containing 2.37% CO_2; 21% O_2; balance N_2. Dynamic compliance and Vrx' were measured as in the capsaicin experiments.

HIBC following airway anaesthesia.

The effect of airway hypocapnia was studied in vagotomized guinea pigs (583 ± 146 gm, mean ± SD) with and without topical airway anaesthesia to determine whether local axonal reflexes might be involved in HIBC. The animals were anaesthetized, tracheotomized and ventilated as described above, however, 15 minutes before cardiac ligation either lignocaine (2%, Astra, n=8), bupivacaine (0.5%, Astra, n=8) or normal saline (n=8) was delivered to the airway surface in the following manner. The animals were disconnected from the ventilator so that 0.3 ml of local anaesthetic solution or normal

saline could be applied directly to the airway using a syringe and fine catheter (0.1 ml each in the right and left main bronchi and 0.1 ml in the trachea). The ventilator was then reconnected, the lungs sighed to a P_L of 39.5 $\pm$ 1.2 cm H_2O (mean $\pm$ SEM) and mechanical ventilation resumed. Further local anaesthetic or saline was delivered as an aerosol to the airways for 200 breaths. The aerosols were generated by a Turret nebulizer (oxygen 8 L/min). Five minutes after the completion of aerosol delivery the heart was ligated and the lungs ventilated with heated (37°C) and humidified (95%) 0% CO_2: 21% O_2: 79% N_2. Dynamic compliance and Vrx' were measured as in the capsaicin experiments.

Statistics.

For experiments comparing two treatment groups an unpaired Student t-test was used to test for a significant difference. Otherwise, one-way analysis of variance and the Bonferroni test (Wallenstein *et al*, 1980) were used to determine whether significant differences existed between group mean values. $P < 0.05$ was considered to be significant.

RESULTS

Lung wet-to-dry weight ratios were routinely calculated at the completion of experiments and found in each animal to be within the previously determined normal range (Chapter 2).

Capsaicin pretreatment.

The effects of capsaicin pretreatment on hypocapnia induced changes in Cdyn and Vrx' are shown in Figures 11A and 11B, respectively. A marked attenuation of the fall in Cdyn was evident at 15 mins in capsaicin treated animals compared with controls. (Figure 11A. Control, Cdyn 55.5 $\pm$ 5.3 % of baseline (mean $\pm$ SE); Capsaicin, Cdyn 85.3 $\pm$ 2.3 % of baseline, $P < 0.0005$). In capsaicin treated animals there was also a marked decrease in gas trapping (Figure 11B. Vrx': Control 5.05 $\pm$ 0.39 mls/gm dry lung; Capsaicin 1.99 $\pm$ 0.39 mls/gm dry lung, $P< 0.0002$). The mean Vrx' for capsaicin treated animals was not different from values found in the previous study (Chapter 2) for lungs immediately excised postmortem and not exposed to hypocapnic gas mixtures (Figure 11B).

Enkephalinase inhibition.

Phosphoramidon treatment significantly potentiated the changes in Cdyn seen in lungs ventilated with a gas mixture containing 2.37% CO_2 (Figure 12A) and there was also a marked increase in gas trapping

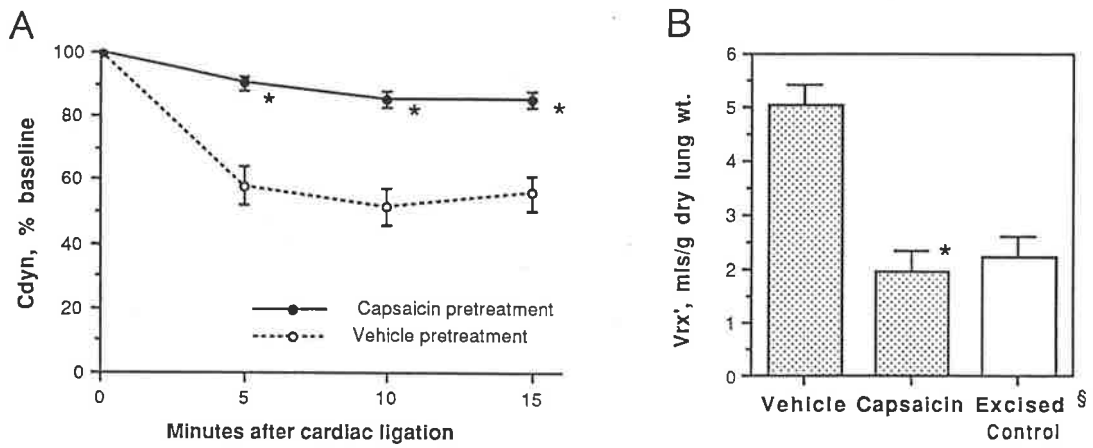


Figure 11. Effect of capsaicin pretreatment on postmortem lung function changes induced by 15 minutes continuous ventilation with an hypocapnic gas mixture (0% CO_2; 21% O_2; 79% N_2, 37°C, 91% humidity). Values are mean ± SEM. * P< 0.0005 compared with vehicle control. (A) Changes from baseline in dynamic compliance (Cdyn). (B) Relaxation lung volume (Vrx') at 15 minutes postmortem. § Vrx' data from earlier study (Chapter 2), in which lungs were excised immediately following cardiac ligation and not exposed to hypocapnia (not significantly different from capsaicin group).

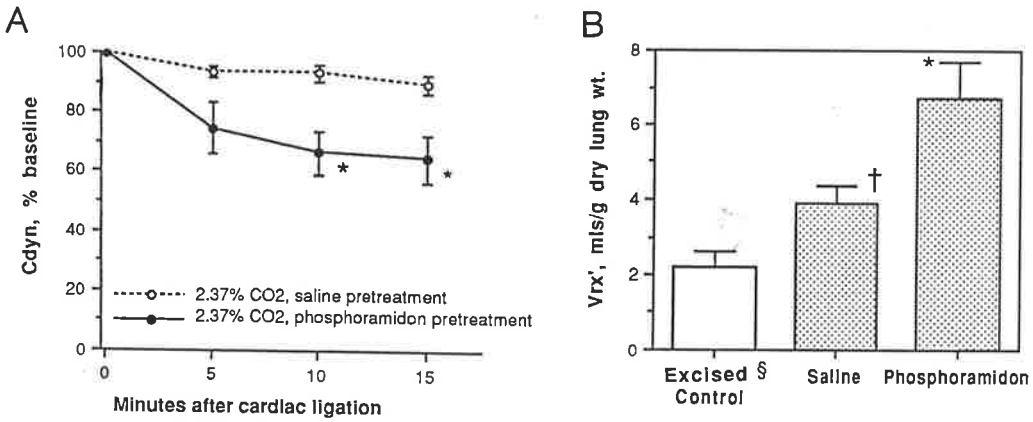


Figure 12. Effect of phosphoramidon infusion on postmortem lung function changes induced by 15 minutes continuous ventilation with an hypocapnic gas mixture (2.37% CO_2; 21% O_2; balance N_2, 37°C, 95% humidity). Values are mean ± SEM. (A) Changes from baseline in dynamic compliance (Cdyn). (B) Relaxation lung volume (Vrx') at 15 minutes postmortem. § Vrx' data from earlier study (Chapter 2), in which lungs were excised immediately following cardiac ligation and not exposed to hypocapnia. * $P < 0.03$ compared with saline control. † $P < 0.02$, compared with excised control.

(Figure 12B. Vrx': Saline Control, 3.90 ± 0.45 mls/gm dry lung; Phosphoramidon, 6.72 ± 0.99 mls/gm dry lung, P< 0.03).

Topical airway anaesthesia.

Lignocaine delayed the hypocapnia-induced fall in Cdyn but did not prevent it. Bupivacaine, on the other, almost completely eliminated hypocapnia-induced changes in Cdyn. (Figure 13A. Control, Cdyn at 15 mins, 65.1 ± 4.0 % of baseline, Bupivacaine, 94.6 ± 3.3 % of baseline, P< 0.05). In the saline treated control group there was marked gas trapping (Figure 13B). Lignocaine decreased Vrx' but the result did not reach statistical significance. Bupivacaine treated lungs trapped significantly less gas (Figure 13B. Vrx', Bupivacaine 2.55 ± 0.27 mls/gm dry lung; Saline Control 4.17 ± 0.48 mls/gm dry lung, P < 0.05).

DISCUSSION

These results confirm the previous findings (Chapter 2, Reynolds and McEvoy, 1988) that airway hypocapnia is a potent stimulus for bronchoconstriction in the postmortem guinea pig lung. Furthermore, the data show that this response occurs in a dose-dependent manner. The major new finding, however, is that HIBC in guinea pig lungs appears to be mediated by TKs, which are released following the activation of a bronchial axonal reflex.

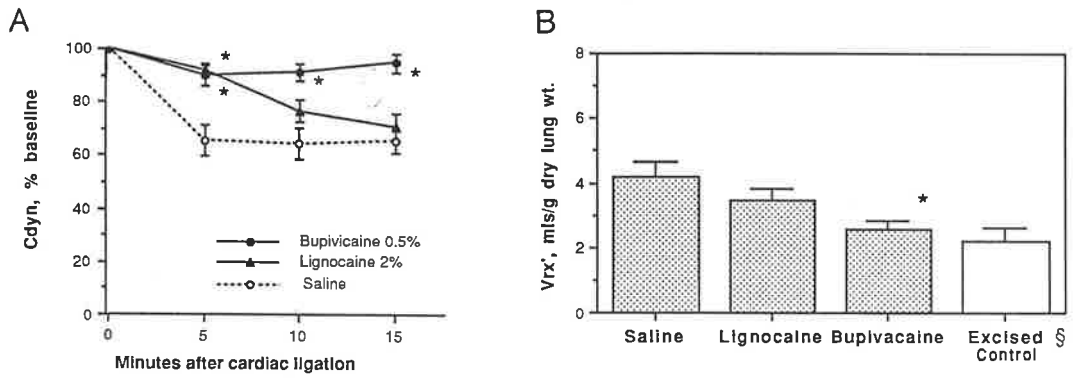


Figure 13. Effect of topical airway anaesthesia on postmortem lung function changes induced by 15 minutes continuous ventilation with an hypocapnic gas mixture (0% CO_2; 21% O_2; 79% N_2, 37°C, 95% humidity). Values are mean ± SEM. * $P < 0.05$ compared with saline control. (A) Temporal changes in dynamic compliance (Cdyn). (B) Relaxation lung volume (Vrx') at 15 minutes postmortem. § Vrx' data from earlier study (Chapter 2), in which lungs were excised immediately following cardiac ligation and not exposed to hypocapnia, (not significantly different from the bupivacaine group).

Three different experimental interventions were used to investigate the possible role of TKs in HIBC. In each experiment Cdyn and Vrx' were employed as indices of bronchoconstriction. While the changes observed following ventilation with hypocapnic gas mixtures (viz. decreased Cdyn and increased Vrx') are consistent with acute bronchoconstriction leading to gas trapping, it is important to consider other possible explanations. For example, pulmonary oedema can also lead to a reduction in Cdyn (decreased lung distensibility) and increased Vrx' (gas trapping due to fluid menisci in small conducting airways). However, this could not explain the present results since all experiments were conducted following circulatory arrest and the wet-to-dry weight ratios of lungs at the completion of experiments were normal. Another theoretical explanation for reduced Cdyn is a decrease in surfactant quantity or function. Under these conditions, however, it would be expected that Vrx' would decrease because of reduced lung distensibility, rather than increase, as was observed. It therefore seems reasonable to assume that the observed changes in Cdyn and Vrx' postmortem were, in fact, due to acute bronchoconstriction.

The results obtained following capsaicin pretreatment stongly implicated TKs in HIBC. It was found that pretreatment with capsaicin over 5 days resulted in a marked attenuation in HIBC, although, a small residual response to airway hypocapnia was still observed. The capsaicin pretreatment regime employed has been shown to markedly, although not completely, deplete peripheral sensory neurons of TKs

(Papka *et al*, 1984). The small residual response that was observed in capsaicin pretreated animals may therefore have been due to the release of persisting TK stores. Alternatively, the residual response could have been due to the release by hypocapnia of other bronchoconstricting mediators from sources such as bronchial epithelium or macrophages. It was also possible that hypocapnia had a direct effect on bronchial smooth muscle tone. While smooth muscle contraction seemed the most likely explanation for the changes observed after airway hypocapnia, TKs also cause bronchial mucous gland secretion (Borson *et al*, 1987), and it was possible that this could also have contributed to acute airway narrowing.

Tachykinins are rapidly degraded in airways by the enzyme enkephalinase - EC 3.4.24.11 (Martling, 1987a). In part this may be because of the close physical proximity of this enzyme to sites of TK release (nerves) and action (smooth muscle) (Sekizawa *et al*, 1987a). It is not surprising therefore that the addition of an enkephalinase inhibitor has been shown previously to augment the affects of TKs (Borson *et al*, 1987, Sekizawa *et al*, 1987a, Sekizawa *et al*, 1987b, Stimler-Gerard, 1987). In the present experiments there was, following phosphoramidon infusion, approximately a twofold increase in gas trapping and an exaggerated decline in Cdyn when lungs were exposed to an intermediate hypocapnic stimulus (2.37% CO_2). These data further strenghten the role for TKs in HIBC.

Tachykinins are located within peripheral non-myelinated afferent

nerves and are released in peripheral sites following the activation of nerve endings and antidromic nerve conduction. This so called "axonal reflex" has been demonstrated in the skin but it has also been proposed as a mechanism for bronchoconstriction and airway inflammation in asthma (Barnes, 1986). Results from the capsaicin and phosphoramidon experiments indicated that hypocapnia had caused TK release within the airways. It seemed likely therefore that hypocapnia, and perhaps mucosal surface pH changes, had activated a bronchial axonal reflex. To further investigate the role of sensory nerves in HIBC, topical anaesthesia was applied to the airway surfaces prior to ventilation with an hypocapnic gas mixture. Lignocaine appeared to partially suppress or delay HIBC, whereas, bupivacaine markedly attenuated the fall in Cdyn and the volume of trapped gas at 15 minutes. The short lived protective effect of lignocaine could have been due to its relatively short half-life. Experiments were not completed until 30 minutes after local anaesthetic was first administered. Enright and colleagues (1980) found in humans that suppression of the gag and cough reflex with 4% lignocaine lasted only 15-20 mins. Bupivacaine on the other hand is a more lipid soluble local anaesthetic with a longer duration of action (Tucker and Mather, 1979). It was not surprising therefore that it had a greater protective effect against HIBC.

Large doses of local anaesthetics have been shown **in vitro** to directly inhibit the contractility of smooth muscle (Fleisch and Titus, 1973). However, this seemed an unlikely explanation for the

attenuation of HIBC following local anaesthetic in the present study as aerosol inhalation in dogs of 5% bupivacaine (10 times the concentration used in this study) blocked nerve transmission without affecting the ability of bronchial smooth muscle to contract with histamine (Dain *et al*, 1975). Local anaesthetic agents are capable of blocking conduction in both efferent and afferent nerves. One cannot therefore entirely exclude some effect of lignocaine and bupivacaine on bronchial efferent nerves. However, local anaesthetic agents are 10 times more effective in blocking small non-myelinated sensory fibres in guinea pig airways than myelinated efferent fibres (Karlsson and Persson, 1984). Furthermore, it was considered that efferent nerve activity was unlikely to have played a significant part in HIBC in this model because both vagi were sectioned in the neck. For these reasons it was considered that attenuation of HIBC by topical airway anaesthesia was most likely due to the inactivation of a bronchial sensory axonal reflex.

This study is the first to implicate TKs in HIBC. However, a number of important questions are left unanswered. The way in which airway hypocapnia led to sensory nerve activation and TK release was not addressed and one can only speculate on the possible mechanisms. Hypocapnia increases excitability in demyelinated nerve fibres (Burchiel, 1981). It is possible therefore that hypocapnia (or accompanying pH changes) could have directly stimulated sensory nerve endings lying within epithelium. Alternatively, hypocapnia might have released other mediators, such as histamine, from cells that were on

or near the airway surface. These mediators could then secondarily have activated sensory nerve endings (Foreman and Jordan, 1983). Coleridge *et al* (1978) showed in dogs an increase in firing rates of pulmonary afferent nerve fibres following exposure of their receptors to low CO_2 concentrations. In their study the most striking effects were observed for pulmonary stretch receptors. Firing rates in C-afferent fibres did not appear to increase. The present data, however, suggested that, in the guinea pig, C-afferent fibres were also affected by changes in airway CO_2 concentrations.

The mechanisms by which TKs produced acute bronchoconstriction in response to hypocapnia are also unknown. Tachykinins are known to have direct spasmogenic effects on bronchial smooth muscle (Martling, 1987a, Martling *et al*, 1987b), but they can also release other chemical mediators from mast cells (Goetzl *et al*, 1985), macrophages (Hartung *et al*, 1986) and possibly eosinophils (Simone *et al*, 1987). Some of these chemical mediators can themselves cause smooth muscle contraction. Another potential effect of TKs was an enhancement of presynaptic release of acetylcholine (ACh) from cholinergic nerves (Tanaka and Grunstein, 1984). However, in the present experiments most vagal efferent nerve activity would have been prevented by vagotomy and recent studies have cast doubt on the role of ACh in HIBC (Jamison *et al*, 1987, Kolbe *et al*, 1987). Finally, the relative contributions of the various different TKs in HIBC remain to be determined. It is quite likely that more than one is involved since at least 3 (substance P, neurokinin A and neuropeptide K) have been

found to co-localize in bronchial sensory nerves (Hau *et al*, 1986, Martling *et al*, 1987b) and each can produce smooth muscle contraction (Martling *et al*, 1987b). It would seem unlikely that calcitonin gene related peptide, which is also found in C-afferent fibres, plays a part, since it does not appear to contract guinea pig bronchial smooth muscle (Ohtsuka *et al*, 1987).

It is interesting to compare the present results with those of Kolbe and colleagues (1987) who recently undertook an extensive, well controlled study of the mechanisms of hypocapnia-induced peripheral airway constriction in dogs. They showed that HIBC in their model was not mediated by prostaglandins or histamine, nor was it influenced by atropine. However, they found that the response exhibited tachyphylaxis and was suppressed by the administration of a calcium antagonist, nifedipine. While it is difficult to draw direct comparisons between their results and those of the present study, their findings are at least consistent with the theory that TKs are major mediators of HIBC. As outlined above, TKs directly cause smooth muscle constriction, and histamine and prostaglandins, while they may be secondarily released, are not required to explain TK related bronchoconstriction. Secondly, prolonged stimulation of pulmonary sensory nerves can lead to the depletion of TK stores (Lundberg *et al*, 1983). This provides one possible explanation for the phenomenon of tachyphylaxis observed by Kolbe and associates (1987) following prolonged airway hypocapnia.

In summary, this study has provided three independent lines of evidence which strongly suggest that HIBC in guinea pigs is mediated by activation of a bronchial axonal reflex and subsequent release of tachykinins within airway walls. Further studies are required to elucidate the exact mechanisms responsible for sensory nerve activation by hypocapnia and to determine what role, if any, other chemical mediators play in this response.

BIBLIOGRAPHY

ANDERSSON, P. (1980):

Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. Allergy. 35:65-71.

BARNES, P. J. (1986):

Asthma as an axonal reflex. Lancet. 1(8475):242-245.

BORSON, D. B., R. CORRALES, S. VARSANO, M. GOLD, N. VIRO, G. CAUGHEY, J. RAMACHANDRAN, AND J. A. NADEL. (1987):

Enkephalinase inhibitors potentiate substance P-induced secretion of $^{35}SO_4$-macromolecules from ferret trachea. Exp. Lung Res. 12:21-36.

BUCK, S. H., P. P. DESHMUKH, H. I. YAMAMURA, AND T. F. BURKS. (1981):

Thermal analgesia and substance P depletion induced by capsaicin in guinea-pigs. Neuroscience. 6:2217-2222.

BURCHIEL, K. J. (1981):

Ectopic impulse generation in demyelinated axons: effects of $PaCO_2$, pH, and disodium edetate. Ann. Neurol. 9:378-383.

COLERIDGE, H. M., J. C. G. COLERIDGE, AND R. B. BANZETT. (1978)

II. Effect of CO_2 on afferent vagal endings in the canine lung. Respir. Physiol. 34:135-151.

CUTILLO, A., E. OMBONI, R. PERDONDI, AND F. TANA. (1974):

Effect of hypocapnia on pulmonary mechanics in normal subjects and in patients with chronic obstructive lung disease. Am. Rev. Respir. Dis. 110:25-33.

DAIN, D. S., H. A. BOUSHEY, AND W. M. GOLD. (1975):

Inhibition of respiratory reflexes by local anesthetic aerosols in dogs and rabbits. J. Appl. Physiol. 38:1045-1050.

DEAL, E.C., JR., E.R. McFADDEN, JR., R.H. INGRAM, JR., R.H. STRAUSS, AND J.J. JAGER. (1979):

Role of respiratory heat exchange in production of exercise-induced asthma. J. Appl. Physiol. 46:476-475.

DRAZEN, J. M., AND F. AUSTEN. (1975):

Pulmonary response to antigen infusion in sensitized guinea pig: modification by atropine. J. Appl. Physiol. 39:916-919.

ENRIGHT, P. L., J. F. McNALLY, AND J. F. SOUHRADA. (1980):

Effect of lidocaine on the ventilatory and airway responses to exercise in asthmatics. Am. Rev. Respir. Dis. 122:823-828.

FARIDY, E.E., AND S. PERMUTT. (1971):

Surface forces and airway obstruction. J. Appl. Physiol. 30: 319-321.

FARIDY, E.E., S. PERMUTT, AND R.L. RILEY. (1966):

Effect of ventilation on surface forces in excised dogs' lungs. J. Appl. Physiol. 21:1453-1462.

FLEISCH, J. H., AND E. TITUS. (1973):

Effect of local anesthetics on pharmacologic receptor systems of smooth muscle. J. Pharmacol. Exp. Ther. 186:44-51.

FOREMAN, J., AND C. JORDAN. (1983):

Histamine release and vascular changes induced by neuropeptides. Agents Actions. 13:105-116.

FRANCO-CERECEDA, A., H. HENNKE, J. M. LUNDBERG, J. B. PETERMANN, T. HOKFELT, J. A. FISCHER. (1987):

Calcitonin gene-related peptide (GCRP) in capsaicin-sensitive substance P-immunoreactive sensory neurons in animals and man: distribution and release by capsaicin. Peptides. 8:399-410.

FRAZER, D.G., P.W. STENGEL, AND K.C. WEBER. (1979):

Meniscus formation in airways of excised rat lungs. Resp. Physiol. 36:121-129.

FRAZER, D.G., AND K.C. WEBER. (1976):

Trapped air in ventilated excised rat lungs. J. Appl. Physiol. 40:915-922.

GOETZL, E. J., T. CHERNOV, F. RENOLD, AND D. G. PAYAN. (1985):
Neuropeptide regulation of the expression of immediate hypersensitivity. J. Immunol. 135:802S-805S.

GOLD, W. M., G-F. KESSLER, AND D. Y. C. YU. (1972):
Role of vagus nerves in experimental asthma in allergic dogs. J. Appl. Physiol. 33:719-725.

HAHN, A., S.A. ANDERSON, A.R. MORTON, J.L. BLACK, AND K.D. FITCH. (1984):
A reinterpretation of the effect of temperature and water content of the inspired air in exercise-induced asthma. Am. Rev. Resp. Dis. 130:575-579.

HARTUNG, H-P., K. WOLTERS, AND K. V. TOYKA. (1986):
Substance P: binding properties and studies on cellular responses in guinea pig macrophages. J. Immunol. 136:3856-3863.

HAU, X-Y., E. THEODORSSON-NORHEIM, E. BRODIN, J. M. LUNDBERG, AND T. HOKFELT. (1986):
Multiple tachykinins (neurokinin A, neuropeptide K and substance P) in capsaicin-sensitive neurons in the guinea pig. Regul. Pept. 13:1-19.

INGRAM, R.H., JR. (1975):

Effects of airway versus arterial CO_2 changes on lung mechanics in dogs. J. Appl. Physiol. 38:603-607.

JAMISON, J. P., P. J. GLOVER, AND W. F. M. WALLACE. (1987):

Comparison of the effects of inhaled ipratropium bromide and salbutamol on the bronchoconstrictor response to hypocapnic hyperventilation in normal subjects. Thorax. 42:809-814.

KARLSSON, J.-A., AND C. G. A. PERSSON. (1984):

Local anesthetics selectively inhibit non-cholinergic neural contractions in guinea-pig airways. Acta Physiol. Scand. 120:469-471.

KOLBE, J., S. R. KLEEBERGER, H. A. MENKES, AND E. W. SPANNHAKE. (1987):

Hypocapnia-induced constriction of the canine peripheral airways exhibits tachyphylaxis. J. Appl. Physiol. 63:497-504.

LAI, Y-L., AND A. F. CORNETT. (1987):

Substance P-inducing massive postmortem bronchoconstriction in guinea pig lungs. J. Appl. Physiol. 62:746-751.

LAI, Y-L., W.J.E. LAMM, AND J. HILDERBRANDT (1984a):

Factors affecting massive postmortem bronchoconstriction in guinea pig lungs. J. Appl. Physiol. 57:692-697.

LAI, Y-L., W.J.E. LAMM, D.L. LUCHTEL, AND J. HILDERBRANDT (1984b):
Massive postmortem bronchoconstriction in guinea pig lungs. J. Appl. Physiol. 56:308-314.

LEMPERT, J., AND P.T. MACKLEM. (1971):
Effect of temperature on rabbit lung surfactant and pressure-volume hysteresis. J. Appl. Physiol. 31:380-385.

LUM, H., AND W. MITZNER. (1985):
Effects of 10% formalin on fixed lung volume and lung tissue shrinkage. A comparison of eleven laboratory species. Am. Rev. Resp. Dis. 132:1078-1083.

LUNDBERG, J. M., A. FRANCO-CERECEDA, X. HUA, T. HOKFELT, AND J. A. FISCHER. (1985):
Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. Eur. J. Pharmacol. 108:315-319.

LUNDBERG, J. M., T. HOKFELT, C-R. MARTLING, A. SARIA, AND C. CUELLO. (1984):
Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. Cell Tissue Res. 235:251-261.

LUNDBERG, J. M., A. SARIA, E. BRODIN, S. ROSELL, AND K. FOLKERS. (1983):

A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea pig. Proc. Natl. Acad. Sci. U.S.A. 80:1120-1124.

MARTLING, C-R. (1987a):

Sensory nerves containing tachykinins and CGRP in the lower airways. Acta Physiol. Scand. 130(Supp 563):5-57.

MARTLING, C-R., E. THEODORSSON-NORHEIM, AND J. M. LUNDBERG. (1987b):

Occurence and effects of multiple tachykinins; substance P, neurokinin A and neuropeptide K in human lower airways. Life Sci. 40:1633-1643.

McCLENAHAN, J.B., AND A. URTNOWSKI. (1967):

Effect of ventilation on surfactant, and its turnover rate. J. Appl. Physiol. 23:215-220.

McLAUGHLIN, R.F., JR., W.S. TYLER, AND R.O. CANADA. (1966):

Subgross pulmonary anatomy of the rabbit, rat, and guinea pig, with additional notes on the human lung. Am. Rev. Resp. Dis. 94:380-387.

NADEL, J.A., AND J.G. WIDDICOMBE. (1962):

Effect of changes in blood gas tensions and carotid sinus pressure on tracheal volume and total lung resistance. J. Physiol. (London). 163:13-33.

NEWHOUSE, M.T., M. BECKLAKE, P.T. MACKLEM, AND M. McGREGOR. (1964):

Effect of alterations in end-tidal CO_2 tension on flow resitance. J. Appl. Physiol. 19:745-749.

NISELL, O.L. (1950):

The action of oxygen and carbon dioxide on the bronchioles and vessels of isolated perfused lungs. Acta Physiol. Scand. 21:1-62.

O'CAIN, C.F., N.B. DOWLING, A.S. SLUTSKY, M.J. HENSLEY, K.P.STROHL, E.R. McFADDEN, JR., AND R.H. INGRAM, JR. (1980):

Airway effects of respiratory heat loss in normal subjects. J. Appl. Physiol. 49:875-880.

OHTSUKA, M., Y. UCHIDA, A. NORURA, Y. YAMASHITA, S. HASEGAWA, AND K. GOTOH. (1987):

The function of capsaicin sensitive nerve in the guinea pig lung. (Abstract) Am. Rev. Respir. Dis. 135:A97.

PAPKA, R. E., J. B. FURNESS, N. G. DELLA, R. MURPHY, AND M. COSTA. (1984):

Time course of effect of capsaicin on ultrastructure and histochemistry of substance P-immunoreactive nerves associated with the cardiovascular system of the guinea pig. Neuroscience. 12:1277-1292.

REYNOLDS, A. M., AND R. D. McEVOY. (1988):

Prevention of abnormal pulmonary mechanics in the postmortem guinea pig lung. J. Appl. Physiol. 64:1322-1326.

SEKIZAWA, K., J. TAMAOKI, P. D. GRAF, C. B. BASBAUM, D. B. BORSON, AND J. A. NADEL. (1987a):

Enkephalinase inhibitor potentiates mammalian tachykinin-induced contraction in ferret trachea. J. Pharmacol. Exp. Ther. 243:1211-1217.

SEKIZAWA, K., J. TAMAOKI, J. A. NADEL, AND D. B. BORSON. (1987b):

Enkephalinase inhibitor potentiates substance P- and electrically induced contraction in ferret trachea. J. Appl. Physiol. 63:1401-1405.

SEVERINGHAUS, J.W., E.W. SWENSON, T.N. FINLEY, M.T. LATEGOLA, AND J. WILLIAMS. (1961):

Unilateral hypoventilation produced in dogs by occluding one pulmonary artery. J. Appl. Physiol. 16:53-60.

SIMONE, C. De., M. FERRARI, G. FERRARELLI, C. RUMI, L. PUGNALONI, AND F. SORICE. (1987):

The effects of substance P on human eosinophil receptors and functions. Ann. N. Y. Acad. Sci. 496:226-232.

SOUHRADA, M., AND J.F. SOUHRADA. (1981):

The direct effect of temperature on airway smooth muscle. Resp. Physiol. 44:311-323.

STEPHENS, N.L., J.L. MEYERS, AND R.M. CHERNIACK. (1968):

Oxygen, carbon dioxide, H+ ion, and bronchial length-tension relationships. J. Appl. Physiol. 25:376-383.

STERLING, G.M. (1968):

The mechanism of bronchoconstriction due to hypocapnia in man. Clin. Sci. 34:277-285.

STIMLER-GERARD, N. P. (1987):

Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea pig lung. J. Clin. Invest. 79:1819-1825.

STRAUSS, R.H., E.R. McFADDEN, JR., R.H. INGRAM, JR., E.C. DEAL, JR., AND J.J. JAGER. (1978):

Influence of heat and humidity on the airway obstruction induced by exercise in asthma. J. Clin. Invest. 61:433-440.

SWENSON, E.W., T.N. FINLEY, AND S.V. GUZMAN. (1961):

Unilateral hypoventilation in man during temporary occlusion of one pulmonary artery. J. Clin. Invest. 40:828-835.

TANAKA, D. T., AND M. M. GRUNSTEIN. (1984):

Mechanisms of substance P-induced contraction of rabbit airway smooth muscle. J. Appl. Physiol. 57:1551-1557.

TUCKER, G. T., AND L. E. MATHER. (1979):

Clinical pharmacokinetics of local anesthetics. Clin. Pharmacokinet. 4:241-278.

WALLENSTEIN, S., C.L. ZUCKER, AND J.L. FLEISS. (1980):

Some statistical methods useful in circulation research. Circ. Res. 47:1-9.

WOHL, M.E.B., J. TURNER, AND J. MEAD. (1968):

Static volume-pressure curves of dog lungs - in vivo and in vitro. J. Appl. Physiol. 24:348-354.