ACUTE INDUCTION OF TRACHEO-BRONCHOCONSTRICTION IN
MORPHINE/CHLORALOSE ANESTHETIZED DOGS: PHYSIOLOGICAL
APPROACH AND PRINCIPLES OF THERAPY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by
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* * * * *

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The respiratory system serves as a functional gas exchanger and as a defense against perturbing antigens that cause a reflex tracheo-bronchoconstriction which affects homeostasis. In concert with the cardiovascular system, the pulmonary system continually adapts and responds to the demands for oxygen by tissues throughout the body, and pathological abnormalities profoundly deteriorate this function. Airways act reflexly to limit the intrusion of irritating air pollutants that trigger smooth muscle reactivity as in many pulmonary diseases. Tracheo-bronchoconstriction is a common sign in respiratory diseases and can be simulated experimentally in animal models in order to study the underlying mechanisms and to find the proper therapeutic approach. Since airways innervation plays a major role in the mechanism of smooth muscle hyperreactivity, this study was designed to evoke tracheo-bronchoconstriction by different stimuli that alter the autonomic nervous control of the respiratory system. In addition, the respiratory and hemodynamic effects of different classes of known bronchodilators were compared by using induced-bronchoconstriction in anesthetized dogs.

Tracheo-bronchoconstriction in morphine/chloralose anesthetized dogs was induced by hypercapnia (5% CO\textsubscript{2}), hypoxia (10% O\textsubscript{2}), or intravenous injection of 0.5 mg/kg bethanechol. After a 15 min period of baseline, each one of 8 dogs was exposed to
both gas mixtures and bethanechol for 10 min each with 15 min recovery periods between exposures and then monitored for 30 min after bethanechol. Respiratory and hemodynamic parameters were recorded after each period of stimulus or recovery and they included tracheal (Tp), airway (Paw), bronchial (Brp), pulmonary artery (Pap), left ventricular end-diastolic (LVEDP) pressures, and heart rate (HR). Calculated parameters include cardiac output (CO), pulmonary vascular resistance (PVR), and pulmonary compliance (PC). In addition, arterial blood and end-tidal gases (PCO₂, PO₂) were monitored and also pH at the same intervals. Hypercapnia caused nonsignificant (p>0.01) increases in HR, Pap, and LVEDP and nonsignificant decreases in PVR without appreciable changes in respiratory parameters. It increased PCO₂ and PO₂ and decreased pH significantly. Hypoxia, on the other hand, had more pronounced hemodynamic effects in which there were significant (p<0.01) increases in HR, Pap, CO, and PVR with nonsignificant increase in LVEDP. Hypoxia caused noticeable and late increase in Tp which was not different (p=0.013) from the baseline but was significant (p=0.009) compared to hypercapnia. No significant (p>0.01) changes were observed in Paw, Brp, and PC due to hypoxia. End-tidal O₂% was decreased significantly. Changes due to abnormal gas breathing were not persistent and all parameters returned to baseline values upon termination of gas and increasing respiration frequency. In contrast, bethanechol had prolonged effects in which there were significant (p<0.01) increases in Pap, LVEDP, and PVR with no changes in HR or CO. Bethanechol significantly increased Tp and Paw without any significant change in Brp and there was a tendency to decrease PC because of elevated Pap and PVR. No changes in blood or end-tidal gases were observed after bethanechol but pH decreased significantly.
The respiratory and hemodynamic effects of three bronchodilators were compared in bethanechol-induced tracheo-bronchoconstriction in 16 dogs divided into 4 groups in which each group received cumulative doses of aminophylline, atropine, terbutaline, or vehicle (saline). Fifteen minutes of baseline were recorded before the injection of 0.5 mg/kg bethanechol for 10 min followed by injection of each bronchodilator or vehicle. A ten minute interval between doses was allowed and the effects were also monitored at 30 and 60 min after the last dose. Each bronchodilator was given in cumulative doses to each group of 4 dogs as follows: 2.5, 5, 10, and 20 mg/kg of aminophylline; 0.02, 0.04, 0.08, and 0.16 mg/kg of atropine; 0.002, 0.004, 0.008, and 0.016 mg/kg of terbutaline. The same respiratory and hemodynamic parameters as measured in the stimuli experiments were recorded or calculated at the baseline, after bethanechol, 10 min after each dose, and 10, 30, and 60 min after the last dose. Atropine after the 2nd dose and aminophylline after the 4th dose increased HR significantly (p<0.006) compared to the vehicle group with no change in the terbutaline group. Atropine, starting at the 1st dose significantly (P<0.006) decreased the bethanechol-elevated Pap, while terbutaline increased it more compared to baseline. There was no change in the aminophylline group. Atropine at the 1st dose and aminophylline after the 4th dose significantly decreased LVEDP after bethanechol but no changes were seen in the terbutaline group. There were no significant changes in CO or PVR in response to any bronchodilator; however, with atropine and terbutaline there was a tendency to decrease the PVR. Atropine at the 2nd dose was superior to other drugs in decreasing the bethanechol-elevated Tp. The percentage decrease of Tp was 65, 47, and 12.6% with atropine, terbutaline, and aminophylline, respectively. Only terbutaline, starting at the 1st dose decreased Paw. Brp did not change in any treatment group. PC
increased only in the terbutaline group at the 3rd dose with no change in other groups. No appreciable changes were observed in blood and end-tidal gases after the three bronchodilators; however, after the first dose of each drug, pH returned to the normal baseline value. These results demonstrate that atropine is very efficacious in reversing tracheo-bronchial spasm induced by bethanechol compared to the other drugs which suggests a dominant control of parasympathetic innervation in the respiratory system.

In conclusion, compared to gas mixtures, bethanechol has the most impressive and prolonged respiratory and hemodynamic effects that resemble acute clinical signs of respiratory diseases such as asthma and COPD. Because the PDE inhibitor and the $\beta_2$ agonist could not antagonize the bethanechol-induced bronchoconstriction, it may be concluded that tonic control of large airways is cholinergic. With some limitations such as anesthetic use and differences among species, this model might be of benefit in evaluating the mechanism of other bronchodilators that alter autonomic nervous control. In the future, particular interest should be directed to new $M_3$-selective antimuscarinics that relax airway smooth muscle and decrease mucus secretion with few or no cardiovascular side effects.
Dedicated to my Parents, Wife, and Family
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

“\textit{The explanation of these striking phenomena has now been found to be this: The Central nervous organ for breathing movements is in a condition of continuous dependence on the prevailing state of lung distention, through the agency of the vagal fibers which terminate in the lung}”

[Breuer and Hering, 1868]

Translated by Ulmann, 1970

1.1 HISTORICAL OVERVIEW:

The respiratory system is one of the vital organs of the living organisms. The direct contact between this system and the external environment allows its primary function of gas exchange to provide oxygen and dispose of carbon dioxide from blood. This intimate relationship exposes the respiratory tract and consequently the whole body to the intrusion of pollutants and other harmful substances in the air. Therefore, this system serves as the first line of defense against changes in the surrounding milieu by bronchoconstriction to limit the entrance of detrimental substances and by expulsion (cough) and mucus secretion. This system is positioned very close to the cardiovascular system in which they usually are referred to as “cardio-pulmonary system”, hence they integrate with each other by providing and distributing exchanged gases to all organs and tissues via blood (Figure 1.1).
The importance of the respiratory system was realized many centuries ago in Greek culture when the old belief was that evil souls and spirits enter through the nose and spread out in the body to cause maladies and illnesses. This myth was extended to be a reality in that breathing is a sign of life which distinguishes living creatures from the inanimate. After several centuries and through the study of anatomy, the first great epoch in the history of respiration was the discovery of blood circulation in the lungs by William Harvey in 1628 (Hutchinson, 1846). In addition, LeGallois in 1812 located a respiratory center in the medulla which later set the frame for other physiologists to uncover a large number of peripheral receptors that supply information to the central control of respiration and reflexly influence breathing (Comroe, 1976). The breakthrough in the history of medicine was the invention of the stethoscope in 1816 by René Laennec, a French physician who examined an obese young lady in labor with heart disease. He could not perform percussion nor was able to listen directly to her heart because of fatness; therefore, he rolled a quire of paper into a cylindrical shape which he placed on her chest and heard a very audible sound (Forbes, 1962). The other remarkable discovery was the Breuer and Hering reflex in 1868 in which they found that if the lung is expanded by inflation, it exerts an inhibitory effect on inspiration and promotes expiration (Ullmann, 1970). The stimulatory effect of carbon dioxide on the chemoreceptors and consequent increase in ventilation was first introduced by Haldane and Priestley in 1905.

After World War II in 1946, clinical pulmonary physiology became one of the primary endeavors of medical science. The first respiratory parameter to be measured was vital capacity (VC) of the lungs by a spirometer that was introduced by Hutchinson in 1946. This device was later improved and became what is known today as the whole
body plethysmograph to measure lung volumes, total lung dynamic compliance ($C_{dyn}$) and resistance ($R_L$), airflow and airway pressure (Paw). The inventions of more sophisticated equipments such as nitrogen meters, strain gages, the infrared analyzer, and oximeters allowed physiological studies to be performed in man. New pulmonary measurements were developed such as lung volume, dead space, alveolar ventilation, and ventilation/perfusion ratio with advances in cardiac catheterization which together became standardized diagnostic techniques in cardiopulmonary diseases (Comroe, 1976). To complement these techniques, blood gas analyzers were invented to measure O$_2$ tension and continuously monitor CO$_2$, which are essential during surgical operations. Thus, pulmonary physiology became an applied science in patient care in every hospital rather than being confined to basic research.

The advancement of neurophysiological studies brought about the recent understanding of the nervous system control of respiration. Most of bronchoconstrictive pulmonary diseases such as asthma and chronic obstructive pulmonary disease (COPD) are thought to be due to abnormal nervous control. Hence, clinical pulmonary physiologists were interested in conducting experiments on animal models that simulate the pathophysiological state in order to find the proper treatment. There is a great interest in this type of research in industrial countries such as England, Germany, United States, Japan, and Canada due to high levels of air pollution and increased population. Although pulmonary research was of interest since the discovery of lung circulation, the golden era of this field started late in the 1950s when it became more applicable to clinical practice and contributed to disease management.
1.2 PULMONARY DISEASES IN HUMAN AND ANIMAL:

1.2.1 Statistical Perspective and Demographical Overview:

Pathological abnormalities and dysfunctions of airways can profoundly affect homeostasis of respiration and consequently the whole body. Lung disease is the number three killer in the United States, responsible for one in seven or about 361,000 annual deaths mostly because of lung cancer (American Lung Association, 2002). There are also chronic diseases that affect more than 25 million people, including asthma, emphysema, chronic bronchitis, chronic obstructive pulmonary disease (COPD), and cystic fibrosis. Asthma is the most prevalent respiratory disease, affecting 5% of the US population which corresponds to 14-15 million individuals and this frequency is comparable to other countries. Although it occurs at all ages, 50% of the cases are noted in children younger than 10 years of age compared to 33% in adults of 40 years or less with males appearing to be about twice as likely to present with asthma as females (McFadden, 2001). Despite the declining mortality of asthma which causes approximately 5000 deaths annually, it costs $14 billion in medical and indirect expenses, 2 million emergency room visits, 500,000 hospitalizations, and 14.5 million missed work days (American Lung Association, 2002). The second important disease is COPD, an umbrella term used to describe chronic bronchitis and emphysema, which together claim the lives of 117,522 Americans annually and cost more than $32 billion in healthcare expenditures. In 2001 an estimated 11 million Americans were diagnosed with chronic bronchitis in which 68.2% are females and 7 million with emphysema in which 57% are males (American Lung Association, 2003).
Unfortunately, there is no accurate estimation of morbidity and mortality of respiratory diseases in animals; yet there are some attempts to report incidences of lower airway disease in dogs and cats as well as COPD in horses, which is equivalent to asthma in human. The limited exercise tolerance and labored breathing in dogs and cats and wheezing in horses after exertion may indicate narrowed airways due to inflammation and bronchoconstriction.

1.2.2 Definitions, Symptoms, and Mechanisms:

Because lung cancer is an uncontrollable disease and eventually leads to death, this review will be limited to chronic diseases, namely asthma and COPD in humans and animals. The other pulmonary diseases such as pneumonia and cystic fibrosis will not be discussed because the former is caused by bacterial infection and sometimes is attributed to underlying conditions, and the latter is a congenital disorder.

1.2.2.1 Asthma:

Asthma is a chronic inflammatory disorder of the airways associated with excessive tracheobronchial reactivity provoked by many stimuli and may be relieved pharmacologically or spontaneously (McFadden, 2001). The manifestations of this inflammation include recurrent episodes of wheezing, chest tightness, and coughing, particularly at night or early morning. These episodes are usually associated with widespread but variable airflow obstruction, especially during expiration, that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli or allergens such as pollens, dust mites, animal dander, or other environmental factors such as cold air, ozone, and sulfur oxide. Asthma is characterized by relatively
asymptomatic periods with occasional acute attack episodes, which may resolve fairly rapidly pursuant to pharmacological intervention but sometimes patients may chronically experience some degree of airway resistance. A serious condition, status asthmaticus, occurs when significant airway obstruction persists for extended periods as opposed to “brittle asthma” which is an unstable condition; the former is defined as a therapeutic emergency. There are many cells and cellular elements that play a major role in this hyperreactivity including mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils, and epithelial cells. An attack of allergic asthma begins when an allergen is inhaled and binds to immunoglobulin E (IgE) antibodies on mast cells in the lungs, which triggers exocytosis of these cells with the release of histamine, leukotrienes, prostaglandins (PGD₂ and PGE₂), bradykinin, and platelet activating factor (PAF). These substances contract smooth muscle of bronchi (bronchoconstriction) as an early phase of the attack (Figure 1.2) and also attract and accumulate other inflammatory cells, especially eosinophils, which cause increased mucus secretion and vasodilation (edema) as a late phase when the lining of bronchi becomes damaged (Figure 1.3). The subcellular mechanism of asthma is more complex in that it starts with the activation of T-lymphocytes, which release lymphokines such as interferon-gamma (INF-γ) and interleukins (IL₄). Interferon-gamma activates macrophages and attracts more leukocytes producing inflammation, while IL₄ stimulates B-lymphocytes to synthesize IgE antibodies that bind to allergens on the mast cells and attracts eosinophils (chemotaxis) which starts the vicious cycle of an asthma attack (Kelley, 1999).

In animals, very similar symptoms and mechanisms can be noticed as in humans; however, this disease is more common in the felines than in the canines in which chronic
bronchitis is more predominant and in the equine with COPD as an equivalent to asthma. Feline asthma was first described in 1906 by Hill when he noticed increased airway mucus, airway inflammation, labored breathing, cough, and wheezing in cats. The clinical signs also include hyperplasia and hypertrophy of goblet and submucosal cells which increase mucus secretion, smooth muscle thickening, and epithelial cells erosion that is associated with eosinophilic infiltration (Padrid, 2000). Pathophysiology of this disease closely parallels that in the human in terms of hyperreactivity and cellular events which trigger bronchospasm, vasodilation, and increased secretions.

1.2.2.2 Obstructive Pulmonary Disease COPD:

Obstructive pulmonary disease is a general term commonly used to describe a group of diseases that cause progressive damage to the lungs such as chronic bronchitis and emphysema which exist together in many patients suffering from irreversible airway obstruction. COPD is considered a chronic, debilitating and sometimes fatal disease in which emphysema can be defined as "alveolar wall destruction with irreversible enlargement of the air spaces distal to the terminal bronchioles and without evidence of fibrosis", whereas chronic bronchitis is defined as "productive cough that is present for a period of 3 months in each of 2 consecutive years in the absence of another identifiable cause of excessive sputum production" (American Thoracic Society, 1995). The most common symptoms and signs are cough, dyspnea on exertion, increased phlegm production, wheezing, prolonged expiration with pursed lip breathing and with the use of accessory muscles of breathing and, in advanced cases, cyanosis, evidence of right heart failure, and peripheral edema. The pathologic hallmark of emphysema is elastin breakdown with resultant loss of alveolar wall integrity which is triggered by the
exposure of a susceptible individual to noxious particles and gases. Cigarette smoke remains the main causative agent, involved in over 90% of cases; however, other gases and particles have been shown to play a role in the pathogenesis, which is due to an inflammatory process. In contrast to the eosinophilic inflammation seen in asthma, the predominant inflammatory cells are neutrophils but also with an increased number of macrophages and T-lymphocytes in various parts of the lungs. Several mediators are released from these cells including leukotriene B4 (LTB₄), interleukin 8 (IL₈), and tumor necrosis factor alpha (TNF-α), which contribute to the inflammatory process (Keatings et al., 1996). Another important factor in the pathogenesis of COPD is oxidative stress which alters protease/antiprotease balance, especially in individuals with severe deficiency of alpha₁-antitrypsin (α₁-AT), which genetically predisposes to emphysema (Repine et al., 1997). The pathophysiology of chronic bronchitis is characterized by increases in the size and number of goblet cells which leads to excessive mucus secretion and the consequence of increased bacterial infections in the bronchial tubes which, in turn, impedes airflow. When COPD is complicated by hypoxemia, intimal and vascular smooth muscle thickening may cause pulmonary hypertension, a late and poor prognostic development in this disease. (Siafakas et al., 1995).

COPD in animals is less defined in that it mostly resembles human asthma in older horses and chronic bronchitis in small to medium size dogs that are in middle age or older (Beech, 1991; and McKiernan, 2000). The clinical signs in horses include heaving to push air out of the lungs towards the end of exhalation, coughing, weight loss, and exercise intolerance, wheezing, and muco-purulent nasal discharge (composed of mucus and inflammatory cells), especially after exercise. The abdominal muscles of COPD
afflicted horses may be enlarged and form noticeable "heave lines", which do not appear to be breed or gender related; however, it may be hereditary. It is caused by an allergic response to the particles in hay dust which contain microorganisms such as bacteria (e.g. *thermoactinomyces vulgaris*) and fungi (e.g. *Aspergillus fumigatus*) as well as tiny particles of feed grains, plants, feces, dander, and pollens which provoke the immunological responses. After 4-6 hours exposure to hay dust, the airways become inflamed and massive numbers of neutrophils accumulate within the air passages in order to eradicate the bacterial infection with the released mediators causing some of the changes in the airway epithelium that are observed in COPD horses. Repeated episodes of inflammation can cause edema and proliferation of the mucosal cells, resulting in a thickening of the airway walls and obstruction of normal airflow during breathing. Inflammation also stimulates mucus secretion and proliferation of goblet cells and the excess of mucus production and cellular debris in the airways plug the bronchioles (Figure 1.3). Inhaled irritants stimulate the parasympathetic nervous system to release acetylcholine (Ach), which causes bronchoconstriction (bronchospasm) to prevent irritants from penetrating deeper into the lungs (Figures 1.2 and 1.4). Since the air passages are obstructed, oxygen cannot be efficiently delivered to the alveoli, which results in a low partial pressure of oxygen in the arterial blood and tissues. This hinders horses from performing normally and results in exercise intolerance. In addition, inflammation damages the epithelium which exposes irritant receptors and nerves become more sensitive to stimuli and, consequently, increase the hyperreactivity of reflex cough.
The definition of chronic bronchitis in dogs was first proposed by Wheeldon and others in 1974 who obtained clinical and pathological signs from 26 dogs that mimic the case in humans. This disease leads to life-threatening disorders and affects medium size dogs of middle age or older, with susceptible breeds such as West Highland White terriers and Cocker Spaniels. It is characterized by chronic cough that lasts for two consecutive months during the preceding year which is not due to other causes such as neoplasia, heartworm, or congestive heart failure (CHF). The inciting cause of this disease is rarely ever determined but it is thought to be induced by various inhaled irritants such as sulfur dioxide. The clinical signs include spontaneous coughing, crackles on auscultation, and excess mucus. The pathological changes include squamous cells metaplasia of tracheobronchial epithelium, increased goblet cells numbers, glandular hypertrophy, and mucosal inflammation (Mckiernan, 2000). Inflammation can cause cellular infiltrates which together with mucosal edema and loss of ciliated epithelial cells contribute to airway narrowing that is reflected as an increase in resistance and decrease in compliance of the lungs. In advanced stages, hypoxemia is evident because of changes in the ventilation/perfusion ratio as well as the irreversible structural changes such as softening of the cartilaginous support of airways known as bronchiectasis.

The resulting clinical signs of these diseases include cough, wheeze, difficulty of breathing, and decreased exercise capacity and are due to airway narrowing (airflow reduction) because of bronchoconstriction and excessive mucus secretion. Therefore, it is necessary to distinguish between these bronchoconstrictive conditions in order to find the proper strategy of treatment and also to rule out the possibility of other comorbid cardiovascular diseases.
1.2.3 Diagnosis and Clinical Respiratory Parameters:

Generally, the diagnostic procedures are quite similar in different types of respiratory diseases which are extrapolated from the human medical practice to the veterinary field. The physician or veterinarian starts with the physical examination of the pulmonary system including inspection, palpation, percussion, and auscultation after knowledge of patient’s history and complaints (Hamlin, 2000). For example, asthma can be recognized by increased respiratory rate and effort during expiration and hyperresonant sounds upon percussion with wheezing, crackles, and hacking cough with auscultation.

Chest X-radiography and the recent advancement in bronchoscopy are very useful techniques that give information about lung parenchyma and conducting airways to assess the severity of bronchoconstriction and exudates as well as to obtain lung biopsy.

Cytological examination of sputum and other airways fluids is very important in differentiating between asthma, in which eosinophils are abundant, and emphysema and chronic bronchitis, where neutrophils and macrophages are predominant (Padrid, 2000). Moreover, sampling of airways secretions can be achieved by transtracheal wash (TTW) or by bronchoalveolar lavage (BAL) during bronchoscopy to determine the bacterial infection.

The most important and routinely prescribed test to diagnose pulmonary diseases is the pulmonary function test (PFT) which can be easily performed noninvasively by using a spirometer to measure how much and how quickly air can be expelled following a deep breath. To determine airflow obstruction, one should measure the ratio of the forced expiratory volume in one second (FEV₁) to the forced vital capacity (FVC). A decrease
of the FEV₁/FVC is characterized as COPD. If this ratio increased by at least 12% and 200 ml after bronchodilator therapy, this means that airflow obstruction is reversible and may indicate asthma. The other vital respiratory parameter in asthma is peak expiratory flow (PEF), the reduction of which reflects the severity of air obstruction in asthma and it is a complement to the FEV₁/FVC test (Jain et al., 1998). These parameters are usually obtained after provoking hyperreactivity by methacholine (or histamine) inhalation in atypical chest symptoms in which there is at least 20% decline in FEV₁ during incremental methacholine aerosolization. In pulmonary physiological studies, a more sophisticated instrument called the plethysmograph is used to measure lung volumes and intrathoracic and transpulmonary pressures in order to assess changes in pulmonary resistance and compliance. Although it is very expensive to use this devise in veterinary medicine, it has recently been employed in the diagnosis of asthmatic cats and in the investigational research settings of new bronchodilators (Hoffman et al., 1999).

To further diagnose these diseases, blood gas analyses are of special importance because they indicate hypoxemic conditions with a severe drop (<80%) of arterial blood oxygen tension (PaO₂). Also, increased PaCO₂ (>45%) reflects abnormal ventilation/perfusion mismatch due to hypoventilation. Arterial blood gas measurement (ABG) is very useful in veterinary medicine because it is easy to perform and it determines the vasoconstriction, as a result of hypoxia, and the potential of developing pulmonary hypertension in canine chronic bronchitis. Another noninvasive measurement is the diffusing capacity of the lungs for carbon dioxide that helps in differentiating between emphysema and chronic bronchitis where decreased diffusing capacity indicates a loss of alveolar-capillary units, suggesting emphysema.
Differential diagnosis should be considered when a decision is to be made concerning respiratory symptoms, since many diseases evoke similar manifestations of cough and chest tightness. Congestive heart failure, for instance, has the same hacking cough as in asthma; however, heart rate increases in CHF because of increased sympathetic tone, while there is pronounced sinus arrhythmia in pulmonary diseases (increased rate with inspiration and slow with expiration) (Hamlin, 2000). Other possibilities that have to be ruled out include mitral valve regurgitation, dilated cardiomyopathy, heartworms, bronchiectasis, pneumonia (allergic, bacterial, fungal), and airways neoplasia.

1.2.4 Management and Treatment:

Since most of the pulmonary diseases are chronic in nature, it should be pointed out that patients must learn to live with and manage these conditions for the long term because they are noncurable disorders. Avoiding many environmental irritants that trigger hypersensitivity is very crucial for asthmatics and quitting smoking is considered a life-saving factor in COPD. Although some respiratory symptoms, such as cough, will not be eliminated completely by medications, it is mandatory that patients follow the course of a therapeutic regimen in a timely manner to control the overall inflammatory process.

The therapeutic approach towards pulmonary disorders is more subjective to relieve the bronchospasm, decrease mucus secretion and suppress inflammation. There are three classical strategies of therapy to achieve reasonable bronchodilation: adrenergic agonists, muscarinic antagonists, and phosphodiesterase (PDE) inhibitors (Figures 1.4 and 1.5). The broad objective of these agents is either to elevate intracellular cAMP ($\beta_2$...
agonists and PDE) or to inhibit the transformation of phosphatidyl 4,5 biphosphate (PIP₂) to inositol 1,4,5 triphosphate (IP₃) and diacylglycerol (DAG) by antimuscarinic drugs (Hoffman and Pappano, 1998). Adrenergic agonists, such as terbutaline, activate smooth muscle β₂ receptors by binding to stimulatory G protein (Gₛ) which activates adenylyl cyclase (AC) that catalyzes ATP to cAMP and promotes relaxation. Presynaptic adrenergic α₂ receptors modify acetylcholine (Ach) release from the parasympathetic limb that innervates smooth muscle. Epinephrine activation decreases Ach release and prevents more bronchoconstriction. Antimuscarinics, such as ipratropium, block M₃ muscarinic receptors on airways smooth muscle which prevents coupling of these receptors to regulatory G protein (G₉/₁₁) and the cascades of increases in intracellular concentration of Ca²⁺ which initiate bronchoconstriction (see innervation section in this chapter). Atropine derivatives also block the presynaptic regulatory M₂ receptors on the parasympathetic nerves which decreases Ach release and curtails bronchospasm. Cyclic adenosine monophosphate is degraded by PDE to the inactive form '5-AMP and this process can be inhibited by PDE inhibitors such as aminophylline, resulting in increased intracellular levels of cAMP and relaxation.

Adrenergic β₂ agonists and PDE inhibitors are commonly used in asthma because these receptors and AC are abundant in the lower, small airways where increased resistance is more profound. In contrast, antimuscarinics are usually used to relieve constriction of central and large airways such as the trachea and main bronchi due to the predominance of cholinergic innervation and increased smooth muscle mass in those areas. In animals, anticholinergics such as ipratropium bromide are drugs of choice in case of chronic bronchitis in dogs and COPD in horses (Mandelker, 2000).
Antimuscarinics are superior to adrenergic agonists in decreasing mucus production from mucosal glands and goblet cells. However, they halt ciliated epithelial cells movement and, consequently, decrease the clearance of sputum which exaggerates airflow obstruction.

The classical treatment of pulmonary diseases with only the bronchodilators mentioned above has many limitations due to several side effects and to the ongoing inflammation cycle that needs to be suppressed. The common side effect is tachycardia resulting from nonselective muscarinic blockers that block cardiac M₂ muscarinic receptors and high doses of β₂ or nonselective adrenergic agonists which activate cardiac β₁ (Brown and Taylor, 2001; and Hoffman, 1998). The desensitization of β₂ receptors upon repeated and chronic activation is another concern that limits the use of adrenergic agonists (Tobias et al., 1990). Moreover, nonselective antimuscarinics in large doses cause dryness of mouth, blurred vision, difficulty in micturition, reduced intestinal peristalsis, and, in severe toxicity, hypotension and CNS side effects such as ataxia, restlessness, excitement, and hallucinations. Nonselective inhibition of PDE by methylxanthines may change the hemodynamic homeostasis such as inotropic effects reflected as increased cardiac output as well as CNS side effects, including hyperexcitement, restlessness, agitations, and tremors. Antiinflammatory agents, such as corticosteroid compounds (prednisolone and fluticasone), have been employed in the long term treatment of asthma to decrease cells infiltration and mediator release (Undem and Lichtenstein, 2001). However, the chronic use of oral glucocorticoids is not recommended due to many side effects that include, but are not limited to, infections (immunosupression), insulin resistance, osteoporosis, myopathy, polyuria, and behavioral
changes. If the cytology results are suggestive of microbial infection such as mycoplasma or candidiasis, antibiotics or antifungals may be prescribed for a certain period of time (Padrid, 2000). It should be emphasized that use of antitussives, such as hydrocodone and dextromethorphan, to calm coughing has very dangerous consequences in productive cough because of sputum accumulation and retention which exaggerates the blockade of airways (McKiernan, 2000).

The new approaches to treatment are aimed more at intervention with the pathogenicity of inflammation and mediator release, including one or two agents from the classic regimen. Antihistamine and antiserotonin agents such as cyproheptadine are useful in blocking the effects of mediators from mast cells, and if they are combined with agents that inhibit mast cells degranulation (stabilizers) such as cromolyn sodium, the outcome will be additive. Recently, specifically designed drugs to block the production of leukotrienes and their binding to receptors are available for clinical practice (Leff, 2000; and Lofdahl et al., 1999). Zafirlukast (ACCOLATE) and montelukast (SINGULAIR) are leukotriene-receptor antagonists, while Zileuton (ZYFLO) is an inhibitor of 5-lipoxygenase, which catalyzes the formation of leukotrienes from arachidonic acid (Undem and Lichtenstein, 2001). Although it is controversial, selective antiprostaglandins or nonsteroidal anti-inflammatory drugs (NSAIDs) are experimentally used, in non-aspirin allergic subjects, to inhibit cyclo-oxygenase-2 (Cox-2) and decrease synthesis of PGD₂, PGF₂, and thromboxane A (TXA) (bronchoconstrictors) while preserving PGE₁₂ and PGI₂ (bronchodilators). Chemotherapeutic agents, such as methotrexate, have experimentally promising results in allergic airway diseases because they suppress cell proliferation by inhibiting folic acid synthesis and they could replace
steroids in asthma or COPD (Domingo Ribas, 1999). The current research interests of finding new drugs includes the anticytokines experimental therapy in asthma, with the examples anti-IL₄ antibodies, anti-TNF-α and anti-INF-γ (Mandelker, 2000). Anti IgE antibodies investigational drugs, such as omalizumab, are another avenue of recent research in treating human asthma. Cloned mouse antibodies can block up to 95% of the mediators release from the mast cells (Milgrom, 2003). Finally, gene manipulation for the treatment of hereditary respiratory disorders such as cystic fibrosis is ongoing. Gene therapy may soon be applicable to decrease the expression of interleukins to prevent airways hyperreactivity.

As discussed earlier, the best regimen treatment for hyperreactive pulmonary diseases should contain a combination of bronchodilators, expectorants, antiinflammatory agents, and mediator release suppressors. More importantly, the use of these medications in veterinary medicine is empirical and based on data extrapolated from human trials which necessitates caution when applied to animals due to intrinsic species differences (Boothe and McKiernan, 1992).
1.3 INNERVATION OF TRACHEO-PULMONARY SYSTEM:

The nervous system is a very complex entity in which there are many components that interact and communicate with each other to maintain homeostasis. The overall control of breathing is achieved by two means: metabolic (automatic) control which is concerned with oxygen delivery and acid-base balance ($\text{PaCO}_2$) and behavioral control (voluntary) which is related to coordinated activities when the breathing pattern is altered such as during speech (Staub, 1998). Normal breathing is controlled centrally by feedback mechanisms (reflexes) between various components of the respiratory system. The signals are carried to respiratory centers in the brainstem (medulla oblongata and pons) which coordinate afferent (sensory) and efferent (motor) neuronal pathways in what is called the reticular formation (King, 1999). It is well known that the whole inflammation process, as a result of hyperreactivity of airways smooth muscle and glands, modulates these pathways which alter normal bronchomotion by increasing muscular tone and glandular secretion to limit the intrusion of triggering antigens. In addition, most of the therapeutic protocols are targeted to resolve the bronchospasm and increased mucus secretion induced by the high excitatory influence of autonomic control of airways (Barnes, 1995).

1.3.1 Autonomic Afferent (Sensory) Innervation of Tracheo-Pulmonary System:

Neural inputs from several sensory receptors in the trachea and lungs, and from other receptors outside the pulmonary system, are carried to the vagus via tracheal and bronchial rami, respectively. Afferent axons from the trachea enter the vagus through the recurrent laryngeal nerve which, along with other axons from bronchial tree, join the main vagal trunk. The cell bodies of all these pathways are in the distal vagal ganglion.
and the central projections proceed through the nucleus of the solitary tract (nucleus tractus solitarius) to the medullary respiratory center in the brainstem (King, 1999). There are two main groups of sensory receptors: mechano-irritant receptors (stretch, irritant, unmyelinated C-fibers) and baro- and chemoreceptors (central and peripheral).

1.3.1.1 Mechano-irritant Receptors (Proprioceptors):

Three main receptors have been identified in the lungs and airways (Table 1.1) which can be stimulated by chemical irritation and distending forces (Widdicombe, 1977; and Coleridge et al., 1989). 1) Slowly-adapting bronchial stretch receptors (SAR) are located in the smooth muscle of the trachea and large bronchi and when stimulates by an increase in bronchial pressure (hyperinflation), they inhibit inspiration and promote expiration (Hering-Breuer reflex). 2) Rapidly-adapting irritant receptors (RAR) are located in the tracheal carina and larger bronchi and ramify among airway epithelial cells in the form of intraepithelial axonal endings which lead into myelinated endings. They are stimulated primarily by noxious agents such as SO₂, NH₄, and inhaled antigens (pollen) and to a lesser extent by mechanical lung inflation and increased airflow. These receptors activate inspiratory motor neurons which cause bronchoconstriction and interact with SAR to increase breathing (rapid, shallow), cough, and mucus secretion in order to limit the penetration of harmful particles. Endogenous mediators resulting from inflammation such as histamine, leukotrienes, and bradykinin also stimulate these receptors. 3) Unmyelinated C-fiber receptors, also known as J-receptors, which exist in the lung interstitium (alveoli) and in the small bronchi, respond primarily to endogenous mediators such as prostaglandins, some inhaled gases and capsaicin. These receptors are excited by various lung pathologies such as pulmonary congestion and edema in which
they reflexly cause apnea followed by tachypnea with bronchoconstriction, mucus secretion, hypotension, and bradycardia. In some species such as guinea pig, there are other afferent fibers called unmyelinated Aδ-fibers which respond to mechanical stimulation and to low pH solutions (Fox et al., 1993). The reflexes of SAR and RAR receptors can be blocked by atropine or vagal cooling while C-fiber reflexes can be blocked by capsazepine.

Airway C-fibers contain several sensory neuropeptides that are considered, by themselves, as the fourth type of mechano-irritant receptors and they were recently identified (Barnes, 1995). These include the neurokinin receptors NK₁ and NK₂ which are activated retrogradely via local (axon) reflex mechanism and have neurotransmitters such as substances P, neurokinin A (NKA), and calcitonin gene-regulated peptide (CGRP). Their release results in microvascular leakage (edema), mucus secretion, and bronchoconstriction which all can be degraded by neuroendopeptidase (NEP) and enkephalinase. The release of sensory neuropeptides may be involved in various models of airway hyperresponsiveness and mediate non-adrenergic non-cholinergic (NANC) bronchoconstriction (see below). These sensory neuropeptides also include neuroepithelial bodies (NEBs) which consist of clusters of respiratory endocrine cells and are embedded in the bronchial and bronchiolar epithelium and alveolar ducts. They are common in neonates and have dense-cored granular vesicles that contain serotonin (5-HT) as the neurotransmitter and when stimulated by hypoxia and hypercapnia, they activate their axonal endings and induce respiratory reflexes through the brainstem.
It has been proposed that the local effect of released serotonin causes pulmonary vasoconstriction, induced by hypoxia or hypercapnia, which is responsible for correcting the perfusion/ventilation ratio by shunting blood from poorly ventilated to unaffected regions.

1.3.1.2 Baro- and Chemoreceptors:

Arterial baroreceptors are located in the carotid sinus, the aortic arch and at the junction of the brachiocephalic and right subclavian arteries. They respond to distention of the arterial walls after a large increase in blood pressure (above 100 mmHg) by reflex adjustment of arterial blood pressure and flow, decrease heart rate, and bronchodilation (Coleridge et al., 1989). The chemoreceptors are found centrally near the ventrolateral surface of the medulla and peripherally in the aortic and carotid bodies which project via aortic and carotid sins nerves, respectively, and join the main vagal trunk to end in the tractus solitarius. They are very sensitive to the changes in concentration of CO₂, O₂, and pH in arterial blood which reflexly increase respiration and decrease blood pressure. Central chemoreceptors are involved in 70-80% of CO₂-induced hyperventilation while peripheral chemoreceptors respond mostly to changes in blood pH and account for 20-30% of increased inspiration in respiratory acidosis when PaCO₂ increased.

Finally, there are afferent inputs from skeletal muscles of the diaphragm, intercostal, and chest and abdominal walls. Receptors in the chest wall include joint, tendon, and muscle spindles, which are located on intrafusal muscle fibers and are innervated by fusimotor fibers or gamma motor neurons. Spindle afferent fibers project to the cerebral cortex and provide information that allows conscious perception of respiratory movements during speech and changes in posture. Tendon organs on intercostal muscles
and the diaphragm monitor force of muscle contraction during inspiration and tend to inhibit it and initiate expiration. These skeletal muscles have nicotinic receptors which are activated by acetylcholine and can be blocked by neuromuscular agents such as tubocurarine and gallamine.

It should be emphasized that 90% of the afferent innervation in the tracheo-pulmonary system is vagal because if the cervical vagus nerves are cut or blocked by atropine, the airways fully dilate and the resistance decreases with no residual airway tone (Coleridge et al., 1989). Moreover, sensory inputs with reflex bronchomotor effects originate not only from endings in upper and lower respiratory tracts, but also from arterial chemo and baroreceptors, and from afferent endings in the heart, skeletal muscle, skin, and gut. These afferent reflexes serve as regulators in response to increased breathing demands such as during exercise and as protectors against irritants by evoking bronchoconstriction and mucus secretion; therefore, sensory inputs are increased from diseased airways.

1.3.2 Autonomic Efferent (Motor) Innervation of Tracheo-pulmonary System:

Motor innervation serves as an “executive” part of the whole feedback mechanisms that arise from afferent inputs and are processed in the central respiratory centers then carried, via efferent nerves, to modulate bronchomotion and mucus secretion. It has been reported that neural mechanisms contribute significantly to the pathophysiology of airways diseases where there is an imbalance between excitatory (parasympathetic) and inhibitory (sympathetic) mechanisms which results from an excessive airway reactivity (Figure 1.4) (Barnes, 1995). To separate afferent innervation of pulmonary system from efferent, one can dissect the recurrent laryngeal nerves (RLN)
and leave the superior laryngeal nerves (SLN) intact; hence no reflex inputs will be conveyed to the central controls (Coleridge et al., 1982). There are three divisions of autonomic efferent control of tracheo-pulmonary system (Table 1.2): cholinergic or parasympathetic, adrenergic or sympathetic, and non-adrenergic non-cholinergic (NANC). These nerves have been reviewed regularly in the literature by many investigators such as Widdicombe, Coleridge, Barns, and Morley; to name a few.

1.3.2.1 Cholinergic Nerves and Receptors:

It is generally agreed that the parasympathetic system has the major influence on airway smooth muscle tone and glandular secretion in humans and animals (Jordan, 2001; Barnes, 1995; Morley, 1994; and Widdicombe, 1985). These nerves originate from the vagal nuclei of the brainstem and pass down the vagus nerve to synapse in parasympathetic ganglia which are situated on the airway walls. From these ganglia, short postganglionic fibers pass to target cells in smooth muscle and glands in the large airways such as the trachea and bronchi; however, few of these fibers can be found in bronchioles and none in the alveolar walls (Barnes et al., 1983). Acetylcholine (Ach) is the neurotransmitter which activates muscarinic receptors in different locations along the parasympathetic fiber. Three types of muscarinic receptors have been identified in the tracheo-pulmonary system: \( M_1 \) on parasympathetic ganglia to facilitate Ach release, presynaptic \( M_2 \) to regulate Ach release (autoreceptor), and postjunctional \( M_3 \) on smooth muscle and mucosal glands. In addition, cotransmitters are released with Ach to inhibit its action such as prostaglandin (PGE\(_2\)) or to potentiate its excitatory effect such as serotonin and thromboxane (TXA). The activation of this limb results in bronchoconstriction and increased mucus secretion which are competitively blocked by
atropine and reversibly blocked by cooling the vagus. Parasympathomimetics such as carbachol, methacholine, and bethanechol are commonly used to activate muscarinic receptors and there are several selective M₁, M₂, and M₃ antagonists (Table 1.2). Activation of M₁ and M₃ results in coupling of these receptors to a regulatory G protein, termed (G₉/₁₁), which stimulates phospholipase C (PLC) to catalyze phosphatidylinositol 4,5 biphosphate (PIP₂) to inositol 1,4,5 triphosphate (IP₃) and diacylglycerol (DAG). IP₃ releases Ca²⁺ from sarcoplasmic reticulum to promote contraction and increase secretion, while DAG activates protein kinase C (PKC) which opens calcium channels and modulates the response (Figure 1.4). On the other hand, M₂ interacts with an inhibitory G protein, termed (Gio), and the resultant inhibition of adenylyl cyclase (AC) and suppression of voltage-gated L-type Ca²⁺ channel activity decrease Ach release (Hoffman and Taylor, 2001).

1.3.2.2 Adrenergic Nerves and Receptors:

There are no direct sympathetic innervations to airways smooth muscle or mucosal glands; however, adrenergic receptors are located abundantly in the smaller airways which are activated by exogenous and circulating catecholamines released from adrenal medulla (Figure 1.4) (Jordan, 2001; Canning and Fischer, 2001; Barnes, 1986; and Widdicombe, 1985). Airway blood vessels and secretory glands do receive direct sympathetic innervations to control blood flow during normal activities and during diseases to correct ventilation/perfusion ratio. Moreover, adrenergic control of airway smooth muscle is weak in normal subjects, depending on the species such as human, while it functions abnormally during asthma (Barnes et al., 1983). In general, there are two types of adrenergic receptors, α and β, that are found on the presynaptic sympathetic
nerve (heteroreceptor or autoregulator) and the postsynaptic effector organ (Table 1.2). Although it is controversial that airway smooth muscle has $\alpha$-adrenergic receptors in some species, they do exist presynaptically on both cholinergic (vagus) and adrenergic nerves as $\alpha_2$ autoregulatory receptors that decrease Ach and norepinephrine (NE), respectively. In addition, postsynaptic $\alpha_1$-adrenoceptors can be found on blood vessels and mucosal glands which upon activation cause pulmonary vasoconstriction and decrease mucus secretion. Nonetheless, there is a controversy on whether $\alpha$-adrenoceptors are important in airway diseases or even exist in normal subjects. Some investigators believe that there are no $\alpha$-adrenoceptors on airway smooth muscle of human (Cabezas et al., 1971; and; Stone et al., 1973); however, these receptors may be quiescent and activated by inflammatory mediators to cause bronchoconstriction such as in asthma (Barnes et al., 1983; and Leff and Munoz, 1981) or indirectly influence airways responsiveness by changing blood flow (Barnes, 1995). The presynaptic $\beta_2$-adrenoceptors are less important in airways because there is no direct sympathetic innervation to the smooth muscle. However, a large number of postsynaptic $\beta_2$ receptors are found on smooth muscle and glands of lower and smaller airways and, in asthma, it is believed that the number is doubled. Activation of $\beta_2$-adrenoceptors relaxes smooth muscle because they are coupled to stimulatory protein ($G_s$) which activates adenylyl cyclase (AC) and consequently increases cAMP (Figure 1.4). Increased cAMP, a second messenger, activates protein kinase A (PKA) which in turn phosphorylates myosin light chain kinase (MLCK) to an inactive form that promotes relaxation (Hoffman, 1998). In contrast, stimulation of $\alpha_1$ results in vasoconstriction but no appreciable bronchoconstriction by activation of PLC and increased intracellular $Ca^{2+}$. Epinephrine activates all types of
adrenoceptors, while NE does not stimulate $\beta_2$ which is selectively activated by terbutaline. There are also several selective and nonselective adrenoceptor blockers that can be used therapeutically as well as in research (Table 1.2).

Despite the fact that the parasympathetic dominates the control of airway bronchomotion, it should be reemphasized that autonomic receptors are distributed heterogeneously along the respiratory tract. Large, central airways (trachea and bronchi) are innervated mostly by cholinergic (vagal) fibers and small and lower bronchioles by adrenergic receptors (Barger et al., 1991; and Banes 1983). In addition, the density of these receptors may differ between species, age, sex, and health condition.

1.3.2.3 Non-Adrenergic Non-Cholinergic Nerves (NANC):

It is usually referred to as the third autonomic nervous system in the lung in addition to cholinergic and adrenergic mechanisms and was identified first in the gastrointestinal tract of many species to control gastric motility and secretion (Barnes, 1984). In 1971, Campbell reported that there are inhibitory nerves which relax toad ($Bufo Marinus$) lungs and were neither adrenergic nor cholinergic. These nerves were proposed after experimental blockade of cholinergic and adrenergic nerves in adrenalectomized and stimulated guinea pigs, where there was a relaxation in airway smooth muscle (Barnes, 1995; and Coburn and Tomita, 1973). Their location and anatomy are hypothetical and it is believed that they are the only dilatory nerves in the human and dysfunction of these nerves may precipitate airways hyperreactivity (Canning and Fischer, 2001; Canning, 1997; Barnes, 1986; and Richardson and Beland, 1976). Although these nerves are less defined, their neurotransmitters may be released concomitantly upon activation of parasympathetic and sympathetic nerves to modulate
the primary functions (Barnes, 1986 and 1995). These neurotransmitters include vasoactive intestinal polypeptide (VIP) and nitric oxide (NO), which are bronchodilators and probably released as cotransmitters with acetylcholine to modify and balance bronchoconstriction (braking mechanism). Relaxation of smooth muscle is attributed to the activation of AC, by VIP, and increased cAMP (same as $\beta_2$ activation). Also, NO activates guanylyl cyclase (GC) and increases the synthesis of cGMP which phosphorylates MLCK and promotes relaxation. There are also excitatory NANC nerves which are believed to accompany afferent C-fibers (see above) and they release their neurotransmitters such as SP, NKA, and CGRP upon the activation of the afferent limb to cause bronchoconstriction, mucus secretion, and edema (Barnes, 1984). The inhibitory neuropeptides of NANC, VIP and NO, can be blocked, experimentally, by $\alpha$-chemotrypsin and L-N$^G$-nitroarginine methyl ester (L-NAME), respectively (Table 1.2). The importance of the NANC could be of particular interest in the near future to develop drugs that enhance inhibitory or suppress excitatory mechanisms in hyperreactive pulmonary diseases.
1.4 METHODS OF EXPERIMENTAL PATHOPHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES OF TRACHEO-PULMONARY SYSTEM:

Since most of the pulmonary diseases result from abnormal autonomic nervous control that leads to increased excitatory mechanisms, studies are more concentrated on the physiology of this system. Studies of the reflex regulation of airway smooth muscle began when Einthoven and others used anesthetized animals to provoke changes in tracheal caliber by CO₂ acting centrally at the level of the medulla (Dixon and Brodie, 1903). Several animal models have been proposed to simulate the pathophysiology of diseases in order to understand the underlying mechanisms and find proper treatment with minimal side effects. In these experimental settings, different stimuli were utilized to induce changes in pulmonary homeostasis including bronchomotion and measurement of vital variables that are reflected as signs and symptoms.

1.4.1 Animal Models:

Because it is difficult to conduct experiments on humans, animals were exploited to study respiratory reflexes and autonomic control of airway smooth muscle. These studies were performed either outside the body on isolated organs and tissues (in vitro), on the intact animal (in vivo), or on an intact isolated organ within the body (in situ). Although many anatomical and histological differences may exist between species, common features of pulmonary system physiology, such as activation of nerves and receptors, may be the same. In vivo and in vitro experiments were done in dog, cat, sheep, pig, rabbit, guinea pig, and rat, while in humans, only noninvasive or in vitro techniques could be performed (Table 1.3). Dogs seem to be more desirable for in vivo studies because of their body size and geometrical position of internal organs as well as their
ability to be trained for experiments using plethysmography. In addition, dogs resemble humans in that they have collateral channels in the lung between alveoli and bronchioles which deliver gases regardless of the airways occlusion (Kraft, 1999). Canine acute models of asthma were used to study the effect of bronchodilators on ventilation/perfusion ratio after methacholine challenge (Rodriguez-Rosin et al., 1984). The canine tracheal pouch with its nerve and blood supply was used as in situ preparation to study tracheal reflex constriction (Allott et al., 1980; and Vidruk, 1985) and to determine neural and humoral factors that control tracheal caliber (Mitchell and Vidruk, 1985). Preconstricted rings of canine bronchi were studied in vitro to examine the local effects of different agents including anesthetics and gases (Lau et al., 1992) or to measure changes in tracheal muscle tension in response to bronchoactive drugs (Filbert et al., 1992).

The advantages of experiments conducted on isolated tracheal segments or strips of airway smooth muscle include avoidance of drug metabolism, effects of anesthetics, and the possibility of monitoring local changes directly. Nevertheless, no reflexes or autonomic controls can be elicited and these organs or tissues represent only part of a whole integrated system with different types of nerves, receptors, and cells. In vitro tests are of value in assessing the overall pharmacological profile of a drug; however, the use of whole animals is more appropriate for the prediction of selective actions in humans (Bowman and Raper, 1976). Intact conscious animals would be the optimal model for any physiological or pharmacological study; however, it is almost impossible and impractical to measure, noninvasively, some of the respiratory and, most importantly, hemodynamic parameters. Therefore, some investigators used decerebrated animals
(Kondo et al., 2000) to completely eliminate any reflex or pain while others use different anesthetic regimens in attempt to maintain normal physiological function. Decerebration technique may alter the respiratory center control and the central chemo- and baroreceptors which modify responses to gases and reduce blood pressure (Iscoe and Fisher, 1995). Some anesthetics, such as pentobarbital, possess the ability to abolish the tracheal constriction reflex (Hosokawa et al., 1984; Allott et al., 1980; and Holtzman, et al., 1982) while volatile anesthetics depress the direct and central effects of CO₂ on airway activity (Lau et al., 1992). The widely used α-chloralose has been evaluated and compared to the effect of other agents and found to be better for preserving reflexes and local effects of other bronchoactive drugs (Jackson and Richards, 1977) as well as maintaining near normal physiological and hemodynamic parameters (Holzgrefe et al., 1987; Silverman and Muir III, 1993; and Zimpfer et al., 1981).

Results of studies that utilized animals as models for pulmonary diseases and treatment should be extended to clinical application with extreme caution, especially in humans, due to species differences and use of anesthetics.

1.4.2 Provocative Stimuli:

Bronchodilation is usually measured as an inhibition of a constant background of bronchoconstriction induced by histamine, acetylcholine, or other stimuli that simulate the signs of a particular disease.

One of the old methods to induce bronchoconstriction is electrical vagal stimulation which excites cholinergic nerve endings on airway smooth muscle to cause contraction and on glands to increase mucus secretion. Vagal stimulation lacks specificity in which it activates both nicotinic and muscarinic receptors as well as weak stimulation
of sympathetic limb and NANC because they are located in the same vicinity. These mixed activations may change many parameters, especially, a decrease in heart rate and drop in blood pressure (Green and Widdicombe, 1966; Sterling at al., 1972; and Szarek, 1989).

Pharmacological induction of bronchoreactivity is widely used to stimulate the afferent limb of autonomic control by capsaicin, serotonin, and histamine or the efferent nerves by parasympathomimetics (see Table 1.3 for references). Aerosolized histamine is a popular agent that has been used in asthmatic animal models (Lunteren et al., 1984); however, it is distributed unequally in the larger more than the smaller airways, which leads to different effects (Ruffin et al., 1978). Moreover, inhaled histamine may irritate airway mucosa and increase, reflexly, the release of other inflammatory mediators besides the direct constrictive effect on smooth muscle (Allott et al., 1980). Cholinergic drugs may be used locally (by inhalation) or parenterally (intravenously); however, some of these agents have nicotinic receptor activities such as methacholine and carbachol which activate ganglia and cause skeletal muscles contraction (Amirav et al., 2001). Acetylcholine is available for intravenous continuous infusion because it is short-lived due to rapid degradation by acetylcholinesterase; yet, it is a nonselective parasympathetic agonist that activates other systems such as the cardiovascular (Kondo et al., 2001-a). Bethanecol, on the other hand, is not susceptible to Ach esterase activity; hence it has longer duration after intravenous bolus injection with no nicotinic activity and minimal cardiovascular effects (Paul, 2002; and Lamid et al., 1982). Administration route of these agents is a very crucial determinant of their effects, onset, and duration of action, since inhaled drug is more directed to the site of action and injected drug is subjected to
metabolism. Intravenous administration is sufficient for distributing drugs evenly through circulation and it is used in experiments concerning hemodynamic effects (Bowman and Raper, 1976).

Another method for inducing tracheo-bronchoreactivity is the use of abnormal concentrations of inspired gas mixtures such as decreased O₂%, hypoxia, and increased CO₂%, hypercapnia. The wealth of information in the literature in regard to the effects of gas mixtures is overwhelming and confusing with controversial and inconclusive results because they may have central, via chemoreceptors, and local direct effects on airway smooth muscle (Loofbourrow et al., 1957). The effect of high CO₂ and low O₂ is bronchoconstriction to different extents depending on concentration, other accompanied stimuli, and different experimental settings (Denjean et al., 1991; and Dickstein et al., 1996). Although the majority of authors agree that hypoxia and hypercapnia bronchoconstrict airways alone or with other stimuli (Dagg et al., 1997; and Yu et al., 1984), few people believe that they cause no significant effects (Hirota et al., 2001; Lau et al., 1992; and Watney et al., 1988) while others suggest that they bronchodilate airways (D’Angelo et al., 2001; Wetzel et al., 1992; and Lindeman et al., 1994). It is well established that CO₂ stimulates breathing via chemoreceptor reflex (Denjean et al., 1991) because of bronchial SAR inhibition, due to decreased pH, which prolongs inspiration (Sant’Ambrogio, 1982). The bronchomotor responses to hypoxia and hypercapnia are mediated by the reflex actions of carotid body and central medullary chemoreceptors, respectively (Coleridge et al., 1989).
Apart from the stimulus type, the effects on tracheo-bronchial smooth muscle depend entirely on the experimental protocols and procedures such as closed or open chest preparations and the surgical interventions that involve anesthetics (see Chapter 2).

1.4.3 Measurement of Tracheo-Pulmonary Changes:

To evaluate the stimuli effects on the functions of the pulmonary system, several parameters can be recorded \textit{in vitro} and \textit{in vivo} using many techniques such as neurophysiological studies, spirometry, plethysmography, radiography, bronchoscopy, and blood gases analysis. In addition, hemodynamic variables, such as heart rate, arterial blood pressure, pulmonary vascular resistance, and cardiac output are important as well because of the close relationship between the respiratory and the cardiovascular systems.

Activity of nerves can be measured using nerve impulse recordings of vagal reflex after applying stimulus. Cooling and atropine blockade techniques are used to inhibit nerve activity, especially that of the afferent to isolate, particularly, feedback mechanisms. Adaptation of SAR and RAR are also measured by recording nerve firing activity in response to lung hyperinflation, while C-fiber activity is recorded after chemical activation by capsaicin, histamine, or bradykinin.

Smooth muscle contraction and bronchoconstriction are reflected as changes in lung compliance and airway resistance using transducers to measure esophageal pressure, as representative of pleural pressure, and intrapulmonary pressure as well as airflow (Coon et al., 1975). Lung compliance is the ability of the alveoli and lung tissue to expand on inspiration to overcome the lung stiffness, and in clinical terms it is defined as the volume increase in the lungs per unit increase in the lung pressure; \( C_L = \Delta V/\Delta P \). There are two types of compliance: static \( (C_{\text{stat}}) \) and dynamic \( (C_{\text{dyn}}) \); the latter is of particular
importance clinically and widely used to express lung stiffness in COPD. Dynamic compliance measurements are made by monitoring the tidal volume used, while intrathoracic pressure measurements are taken during the instance of zero airflow that occurs at the end of inspiratory and expiratory levels with each breath. Furthermore, compliance is derived from the volume-pressure curve in which dynamic compliance measurements are usually performed and the relationship is approximately linear and a constant compliance is assumed. Airway resistance relates to the ease with which air flows through tubular respiratory structures and it is the relationship between changes in airflow to a change in pressure; $R_{aw} = \Delta P/\Delta V$. Pressure differences between airway opening and alveolar ($P_{ao}-P_{alv}$) can be measured by pneumotachograph and the airflow by spirometer and the slope of pressure-flow curve represents lung resistance, which can be directly measured in man by the plethysmograph. As pressure in the thoracic cavity becomes more negative, the airways are widened and the resistance is lowered; conversely, during expiration, when the pressure in the thorax becomes positive, the airways are narrowed and resistance is increased.

These indirect assessments of airway caliber are less sensitive to changes in smooth muscle motion; for example, airway resistance may decrease at a given lung volume if there is alveolar collapse and a decrease in compliance even if the smooth muscle is unchanged (Green and Widdicombe, 1966). Moreover, airway resistance may increase due to other factors such as increased mucus secretion and congestion which are not related to airway smooth muscle contraction. Therefore, direct measurement of tracheal pressure is a good and more sensitive index of airway smooth muscle activity compared to total lung resistance (Ishikawa et al., 1998). This pressure can be directly
measured by an endotracheal cuffed tube from which a small tube connects the cuff to a very sensitive transducer which is consequently connected to a polygraph. Inflating the cuff to a baseline pressure of 15-20 mmHg ensures an optimal contact with the tracheal wall and is taken as a reference pressure when changes occur after applying bronchoactive agents. *In vitro* and *in situ* techniques have been employed to estimate smooth muscle tension in response to many stimuli by attaching stripped muscle fibers to a transducer. This method is beneficial in studies involved in the local effects of different bronchoactive agents; however, it is very difficult to apply this technique to distal and smaller airways due to decreased density of smooth muscle with subsequent arborization of the pulmonary tree.

More expensive techniques to measure bronchoactivity include chest radiography and bronchoscopy that require special preparations such as tantalum powder inhalation or catheter insertion which may irritate airways. Cross sectional pictures of the airways are useful in clinical diagnosis more than in experimental settings because of normal rhythmic contraction of airways that should be monitored continuously (Kondo et al., 2001-b). To measure resistance of parenchyma, some investigators use a device called alveolar capsule which has a transducer placed on the outer pleural surface (Kraft, 1999).

The hemodynamic parameters can be measured invasively in intact animal preparations and they include heart rate, arterial blood pressure, pulmonary artery pressure, left ventricular pressure, and cardiac output. Catheterization is required to measure cardiac output and pulmonary artery pressure simultaneously by a Swan-Ganz catheter inserted through the jugular vein to the right ventricle and ending in the pulmonary artery. Left ventricular and aortic pressures are measured instantaneously by a
Millar catheter inserted through the carotid artery and heart rate is monitored by recording ECG. It is very crucial to monitor blood PO$_2$, PCO$_2$ and pH as well as end-tidal gases since these parameters are distorted by bronchoconstriction and may indicate pulmonary shunt that is reflected as a change in ventilation/perfusion ratio.

1.4.4 Rationale and Specific Objectives:

The rationale of inducing acute tracheo-bronchoconstriction is to simulate an acute asthmatic episode that is encountered in emergency cases and includes respiratory and hemodynamic changes. Three stimuli of hypercapnia, hypoxia, and bethanechol will be used to induce tracheo-bronchomotion in morphine/chloralose anesthetized dogs with minimal or no surgical interventions. It is generally accepted that decreasing PaO$_2$ to a mean of 37 mmHg and increasing PaCO$_2$ to a mean of 66 mmHg will decrease the volume of isolated tracheal segment by approximately 12% and increasing total pulmonary resistance by 50-60% (Nadel and Widdicombe, 1962). However, it would be helpful to assess the tracheal smooth muscle activity by measuring intratracheal pressure which reflects bronchomotion in response to different stimuli.

Direct, continuous, and minimally invasive measurements of the tracheal and bronchial pressures will be performed to assess changes in airway caliber as established by many researchers (Roberts et al., 1988; and Fisher et al., 1995). Although, changes in the tracheal pressure cannot represent the whole airway smooth muscle activity, it is well known that cholinergic innervation and its effects are more dominant in central than in peripheral airways (Russell, 1978). Therefore, using parasympathomimetic agents should achieve satisfactory tracheo-bronchoconstriction that lasts longer than gas mixtures effects.
Objectives:

1) Acute induction of tracheo-bronchoconstriction in the intact, closed-chest animal to preserve normal physiological conditions.

2) Use of an anesthetic protocol that maintains close to normal autonomic nervous control of the respiratory and cardiovascular systems (see above).

3) Comparison of the respiratory and hemodynamic effects of hypercapnia, hypoxia, and bethanechol to identify a reliable and reproducible stimulus that lasts for a considerable period of time and resembles the clinical sings of respiratory diseases.

4) Study of the respiratory and cardiovascular effects of three different classic bronchodilators (atropine, terbutaline, and aminophylline) on a prolonged bronchoconstriction model.

5) Find the best bronchodilator with an optimal dose that relieves bronchoconstriction with less cardiovascular effects in dogs.

6) Reconfirm the heterogeneity of autonomic receptor distribution in large and central versus small airways.

The outcome of this work will shed light on the clinical evaluation of therapeutic bronchoactive drugs, especially the potential use of selective parasympatholytics in COPD and asthma.
Figure 1.1: The Cardio-Pulmonary System.
Figure 1.2: Bronchoconstriction or Bronchospasm.

Figure 1.3: Bronchoconstriction in Asthma and COPD.
Figure 1.4: Innervation and Different Mediators of Bronchial Smooth Muscle and Cellular Events:
Figure 1.5: Mechanism of Actions of Different Bronchodilators in Airways Smooth Muscle:
Note that $\alpha_2$ adrenergic agonists inhibit Ach release from parasympathetic nerve. Abbreviations are same as Fig 1.4.
<table>
<thead>
<tr>
<th>Type</th>
<th>Nerve</th>
<th>Receptor</th>
<th>Stimulus</th>
<th>Transmitter</th>
<th>Reflex or Effect</th>
<th>Antagonist or Blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Afferent (Sensory)</strong></td>
<td>Vagus recurrent laryngeal nerve (superior laryngeal nerve SLN)</td>
<td>myelinated SAR</td>
<td>stretch inflation</td>
<td>acetylcholine</td>
<td>bronchodilation</td>
<td>cooling atropine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>myelinated RAR</td>
<td>irritants inflammation</td>
<td></td>
<td></td>
<td>cooling atropine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unmyelinated C-fiber (J receptor)</td>
<td>inflammation capsacin bradykinin</td>
<td></td>
<td>bronchoconstriction</td>
<td>capsazepine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>myelinated Aδ fiber</td>
<td>mechanical low pH</td>
<td></td>
<td></td>
<td>cooling atropine</td>
</tr>
<tr>
<td><strong>Sensory Neuropeptides</strong></td>
<td>C-fiber or Neuroepithelial bodies (NEBs)</td>
<td>NK₁</td>
<td>retrograde activation of local (axon) reflex</td>
<td>substance P (SP)</td>
<td>microvascular leakage (edema) mucus secretion by goblet cells</td>
<td>neuroendopeptidase (NEP) enkephalinase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NK₂</td>
<td></td>
<td>neurokinin A (NKA)</td>
<td>bronchoconstriction edema, hyperemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>calcitonin gene-related peptide (CGRP)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1: Afferent (sensory) Innervation of Tracheo-Pulmonary system:
NKA and SP are tachykinins. Afferent receptors from chemo and baro receptors are not included because they are located outside the pulmonary system, centrally in medulla and peripherally in aortic and carotid bodies (see text for other afferents).
<table>
<thead>
<tr>
<th>Type</th>
<th>Nerve</th>
<th>Receptor</th>
<th>Transmitter</th>
<th>Agonist or Stimulus</th>
<th>Effect</th>
<th>Antagonist or Blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasympathetic (cholinergic)</td>
<td>muscarinic M₃</td>
<td></td>
<td>acetylcholine</td>
<td>carbachol methacholine bethanechol</td>
<td>constriction and increased mucus</td>
<td>zamifenacin, tiotropium atropine</td>
</tr>
<tr>
<td></td>
<td>presynaptic M₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>trinitramine, atropine</td>
</tr>
<tr>
<td></td>
<td>ganglionic M₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>prazosin, butoxamine</td>
</tr>
<tr>
<td>Efferent Sympathetic (adrenergic)</td>
<td>β₂</td>
<td></td>
<td>epinephrine and epinephrine (circulating catecholamines)</td>
<td>epinephrine terbutaline abuterol</td>
<td>dilation and decreased mucus</td>
<td>prazosin</td>
</tr>
<tr>
<td></td>
<td>α₁ ??</td>
<td></td>
<td>epinephrine norepinephrine phenylephrine methoxamine</td>
<td>epinephrine norepinephrine clonidine</td>
<td>constriction ??</td>
<td>yohimbine</td>
</tr>
<tr>
<td></td>
<td>presynaptic α₂</td>
<td></td>
<td>epinephrine norepinephrine phenylephrine methoxamine</td>
<td>epinephrine norepinephrine clonidine</td>
<td>decrease Ach release (vagus)</td>
<td>propranolol</td>
</tr>
<tr>
<td></td>
<td>presynaptic β₂ ??</td>
<td></td>
<td>epinephrine</td>
<td>epinephrine</td>
<td>increase NE release</td>
<td>propranolol</td>
</tr>
<tr>
<td>Non-adrenergic Non-cholinergic (NANC)</td>
<td>unknown</td>
<td></td>
<td>vasoactive intestinal polypeptide (VIP)</td>
<td>chemical, mechanical, and electrical stimulation</td>
<td>bronchodilation</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>nitric oxide (NO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.2: Efferent (motor) Innervation of Tracheo-Pulmonary system: (L-NAME) L-NG-nitroarginine methyl ester. (??) Controversial or less important.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Author/Years + Other agents</th>
<th>Route of Administration</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>Shiozaki et al, 2000</td>
<td>in vitro</td>
<td>guinea pig</td>
</tr>
<tr>
<td></td>
<td>Cardell et al, 1998</td>
<td>intravenous</td>
<td>guinea pig</td>
</tr>
<tr>
<td></td>
<td>Lunteren et al, 1984</td>
<td>inhalation</td>
<td>human</td>
</tr>
<tr>
<td></td>
<td>Ruffin et al, 1978</td>
<td>inhalation</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Jackson and Richards, 1977</td>
<td>inhalation</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Kondo et al, 2000-a</td>
<td>intravenous</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Lau et al, 1992</td>
<td>5HT</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Micheletti et al, 1987</td>
<td>intravenous</td>
<td>guinea pig</td>
</tr>
<tr>
<td></td>
<td>Sterling et al, 1972</td>
<td>intravenous</td>
<td>dog</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Sterling et al, 1972</td>
<td>intravenous infusion bolus</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Dagg et al, 2001</td>
<td>intravenous infusion</td>
<td>pig</td>
</tr>
<tr>
<td></td>
<td>Eckmann, 2000</td>
<td>intravenous infusion</td>
<td>human</td>
</tr>
<tr>
<td></td>
<td>Clayton, 1999</td>
<td>intravenous bolus</td>
<td>rat</td>
</tr>
<tr>
<td></td>
<td>Dagg et al, 1997</td>
<td>intravenous infusion</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Denjean et al, 1991</td>
<td>intravenous infusion</td>
<td>sheep</td>
</tr>
<tr>
<td></td>
<td>Breen et al, 1987</td>
<td>intravenous infusion</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Rodriguez-Roisin et al, 1984</td>
<td>inhalation</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Yu et al, 1984</td>
<td>inhalation</td>
<td>cat</td>
</tr>
<tr>
<td></td>
<td>Kelsen et al, 1979</td>
<td>inhalation</td>
<td>human</td>
</tr>
<tr>
<td>Methacholine</td>
<td>Hirota et al, 2001</td>
<td>intravenous infusion bolus</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Amirav et al, 2001</td>
<td>intravenous bolus</td>
<td>pig</td>
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<td>intravenous infusion</td>
<td>human</td>
</tr>
<tr>
<td></td>
<td>Eckmann, 2000</td>
<td>intravenous infusion</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Clayton, 1999</td>
<td>intravenous infusion</td>
<td>rat</td>
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<tr>
<td></td>
<td>Dagg et al, 1997</td>
<td>inhalation</td>
<td>human</td>
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<td></td>
<td>Denjean et al, 1991</td>
<td>inhalation</td>
<td>sheep</td>
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<tr>
<td></td>
<td>Breen et al, 1987</td>
<td>intravenous infusion</td>
<td>dog</td>
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<td>Rodriguez-Roisin et al, 1984</td>
<td>inhalation</td>
<td>dog</td>
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<tr>
<td></td>
<td>Yu et al, 1984</td>
<td>inhalation</td>
<td>cat</td>
</tr>
<tr>
<td></td>
<td>Kelsen et al, 1979</td>
<td>inhalation</td>
<td>human</td>
</tr>
<tr>
<td>Carbachol</td>
<td>Hirota et al, 2003</td>
<td>in vitro</td>
<td>guinea pig</td>
</tr>
<tr>
<td></td>
<td>+ Ca** channel blockers</td>
<td>inhalation</td>
<td>cat</td>
</tr>
<tr>
<td></td>
<td>Hirt et al, 2003</td>
<td>inhalation</td>
<td>sheep</td>
</tr>
<tr>
<td></td>
<td>Seuri et al, 2002</td>
<td>intramuscular</td>
<td>guinea pig</td>
</tr>
<tr>
<td></td>
<td>Ben-Jabria et al, 1999</td>
<td>in vitro</td>
<td>dog</td>
</tr>
<tr>
<td></td>
<td>Bazán-Perkins et al, 1998</td>
<td>inhalation</td>
<td>dog</td>
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<td>Strohl et al, 1987</td>
<td>intravenous</td>
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<td>Mielens, 1979</td>
<td>intravenous</td>
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<td></td>
<td>Minatoya, 1978</td>
<td>intravenous</td>
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<td></td>
<td>+ isoproterenol and salbutamol</td>
<td>in vitro</td>
<td>guinea pig</td>
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<td>Spilker and Minatoya, 1975</td>
<td>in vitro</td>
<td>rat</td>
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<td></td>
<td>+ isoproterenol and isoetharine</td>
<td>in vitro</td>
<td>rat</td>
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<td>Bethanechol</td>
<td>Szarek and Spurlock, 1997</td>
<td>in vitro</td>
<td>rat</td>
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<td>Barger and Evans, 1991</td>
<td>in vitro</td>
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<td>Szarek, 1989</td>
<td>in vitro</td>
<td>rat</td>
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<td></td>
<td>+ Serotonin (5HT)</td>
<td>in vitro</td>
<td>guinea pig</td>
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<tr>
<td></td>
<td>Micheletti et al, 1987</td>
<td>in vitro</td>
<td>guinea pig</td>
</tr>
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</table>

Table 1.3: Some Pharmacological Agents Used to Induce Bronchoconstriction in Different Subjects.
1.5 LITERATURE CITED:


60. Keatings, V.; Collins, P.; Scott, D.; and Barnes, P. Differences in Interleukin-8 and Tumor Necrosis Factor-alpha in Induced Sputum from Patients with Chronic Obstructive Pulmonary Disease or Asthma. American Journal of Respiratory and Critical Care Medicine, vol. 153, pp 530-534, 1996.


CHAPTER 2

ASSESSMENT OF THE TRACHEO-BRONCHIAL SYSTEM REACTIONS AND HEMODYNAMIC EFFECTS OF DIFFERENT ACUTE BRONCHOCONSTRICTIVE STIMULI IN MORPHINE/CHLORALOSE ANESTHETIZED DOGS

2.1 INTRODUCTION:

Knowledge concerning the effects of various factors that alter the homeostasis of the respiratory system is being continuously developed by many investigators. One of the most important tools that aid in this type of research is providing a reproducible model that resembles the clinical symptoms and signs of specific respiratory diseases. The common sign of lung and airways diseases, such as asthma and emphysema, is change in the bronchomotor tone, namely bronchoconstriction, in response to different provocative substances.

The earliest attempts to produce tracheal constriction were introduced during the early years of 1900. Loofbourrow and his colleagues, in 1957, reported that there are spontaneous, slow, and rhythmic tracheal constrictions in the anesthetized dog that could be eliminated by vagotomy, hyperventilation, and atropine. These contractions were induced by asphyxia, vagal stimulation, and hypoventilation, and have a direct relation to concentrations of CO₂ in the inspired air. These investigators concluded that the response to hypercapnia depends on the vagal innervation of the trachea and this response could be abolished by stretching the lungs through hyperventilation which relaxed the trachea.
reflexively. Furthermore, they assumed that the trachea is considered as a part of the dead space whose volume is reduced when the ventilation is inadequate to prevent hypercapnia. Moreover, changes in the lung and airways resistance to airflow and compliance, as indicators of bronchoconstriction, are used to diagnose respiratory diseases. However, these indirect assessments are not conclusive of the smooth muscle activity because airway resistance may decrease due to other factors, such as alveolar collapse and increased lung elastance, even if the smooth muscle is unchanged (Green and Widdicombe, 1966). Therefore, the direct measurement of the smooth muscle activity is a more reliable method that represents the actual changes in pressure parameters inside airways (Widdicombe, 1966).

The studies of the smooth muscle control of the airways caliber in response to different agents are still inconclusive and contradictory. These disagreements are due, in part, to the methodologies applied to different preparations such as \textit{in vivo} vs. \textit{in vitro} studies. The indirect measurement of the volume/pressure relationship as a representative of the tracheo-bronchial smooth muscle activity is another factor in this contradiction, as mentioned above. Even in the direct assessment of airway pressures and, consequently, the smooth muscle tone, there are other factors that alter the physiological integrity of the tracheo-pulmonary system. These factors include: surgical procedures (open vs. closed chest), anesthesia (type and depth), and agents used to induce a reaction (concentrations, route of administration, and time of exposure). Examples of these experimental conditions are the anesthetics pentobarbital, which inhibits the vagal component of histamine-induced bronchoconstriction (Jackson and Richards, 1977; and Holtzman et al., 1982), and halothane, which depresses the hypocapnic bronchoconstriction after
cholinergic agonists (Lau et al., 1992). To avoid the complications of anesthesia, some investigators utilized the decerebrated animal as a model to study induced bronchomotor activities (Iscoe and Fisher, 1995; and Kondo et al., 2000-a). However, this technique altered the respiratory center control and the central chemo- and baroreceptors as evident by the reduction in blood pressure and increased sensitivity to the effect of gas mixtures, as reported by the authors.

The area of conflict in the previous works was the inconsistent effects of the inhalation of various gas mixtures in different preparations and species. For example, Green and Widdicombe (1966) found that the response of tracheo-bronchial activity to different gas mixtures was not the same in vagotomized vs. intact dogs or open vs. closed chest. The majority of researchers concluded that gas mixtures, especially hypoxia, produced reversible bronchoconstriction or at least enhanced the effects of other stimuli (Dagg et al., 1997; Yu et al., 1984; Tam et al., 1985; Ahmed and Marchette, 1985; and Denjean et al., 1991). On the contrary, quite a few people believed that there were no significant or specific changes to the tracheo-bronchial tone upon the exposure to gas mixtures, especially hypercapnia (Watney et al., 1988; Coon et al., 1975; Mitchell and Vidruk, 1987; Hilberman et al., 1976; and Hirota et al., 2001). Nonetheless, few investigators suggested that hypercapnia and hypoxia caused, rather, bronchodilation and relaxed the airways smooth muscle (Lau et al., 1992; D’Angelo et al., 2001; Lindeman et al., 1994; and Wetzel et al., 1992).

Most of the aforementioned studies used different respiratory stimuli to induce tracheo-bronchial response in various preparations and species. These stimuli include vagal nerve stimulation, electrically or pharmacologically, which involves very weak
sympathetic activation and consequently alters the heart rate (Green and Widdicombe, 1966; and Kondo et al., 2000-a&b). The nonspecific activation of muscarinic and nicotinic receptors, due to the massive release of acetylcholine, would cause undesirable cardiovascular effects. The pharmacologic induction of bronchoconstriction by histamine inhalation is commonly used through aerosol delivery (Lunten et al., 1984); however, it is deposited into large more than small airways, which causes a non-uniform effect (Ruffin et al., 1978). Inhalation, by itself, would irritate the airways mucosa, which in turn would release more inflammatory mediators and cause other side effects due to the lack of selectivity. Histamine, if injected, may alter the response of the airways due to the generalized vasodilatation and edema that decrease the lung compliance and increase resistance (Cardell et al., 1998). The other injectable parasympathomimetics such as methacholine and carbachol should be used in conjunction with paralyzing agents to prevent nicotinic receptor activation, which in turn inhibits the skeletal muscle of diaphragm and intercostal muscles (Breen et al., 1987; and Amirav et al., 2001). Acetylcholine also has been used in some studies; however, it should be infused over the whole period of experiment due to the rapid degradation by acetylcholinesterase (Sterling et al., 1972; and Kondo et al., 2000-a). Bethanechol, on the other hand, has less or no effect on the nicotinic receptors and, hence, fewer cardiovascular effects. It has been used in in vitro studies to constrict bronchial smooth muscle of rats (Szarek, 1989; and Szarek et al., 1997) and guinea pigs (Micheletti et al., 1987). The present study is the first attempt to apply bethanechol in intact dogs to induce acute respiratory and hemodynamic effects that emulate the tracheo-bronchial constriction seen in asthmatics. The choice of bethanechol was based on the fact that a single intravenous injection gives the desirable
level of tonic and persistent bronchoconstriction with fewer and non-specific cardiovascular effects (Lamid et al., 1982). The onset of its action is 5-15 minutes after subcutaneous injection, peaks within 30 minutes and persists for 2 hours (Paul, 2002). Most of the models that resembled acute asthma have exploited the continuous intravenous infusion of methacholine (Rodriguez-Roisin et al., 1984; and Breen et al., 1987).

2.1.1 Objectives and Hypothesis:

The aim of the experiments in this chapter is to find a reproducible and extended induction of bronchoconstriction by different stimuli in intact morphine/chloralose anesthetized dogs. In addition, comparisons between different stimuli (gas mixtures vs. pharmacological vagal stimulation) are conducted to assess the hemodynamic and respiratory changes in response to a particular stimulus. The hypothesis to be tested is that the induction of tracheo-bronchial constriction by bethanechol, a muscarinic receptor agonist, persists for a longer period of time and is more reliable than hypercapnia or hypoxia with minimum changes in hemodynamic parameters in morphine/chloralose anesthetized dogs. The outcome of these experiments could be of importance in testing the therapeutic agents of bronchodilation that are currently used or will be developed in the future.
2.2 MATERIALS AND METHODS:

2.2.1 Animals:

In this study, all dog experiments were approved by the Institutional Laboratory Animal Care and Use Committee (ILACUC) of The Ohio State University. Eight, young, mature, healthy beagle dogs of either sex with body weight between 8.5 to 14.5 kg (mean ± S.D 11.6 ± 2 kg) were used. Before starting experiments, all animals were checked to ensure that is no evidence of preexisting cardiovascular or broncho-pulmonary diseases.

2.2.2 Anesthesia, Intubation, and Ventilation:

A morphine sulfate/chloralose anesthetic protocol was followed in all experiments due to the fact that this regimen has minimal effects on the cardiovascular system as well as maintains close to normal autonomic control of the respiratory system (Zimpfer et al., 1981; Holzgrefe et al., 1987; and Jackson & Richard, 1977). To alleviate pain and discomfort, dogs first were pretreated intravenously with 1.5 mg/kg morphine sulfate (ELKINS-Sinn, Cherry Hill, NJ) injected over 10 min via cephalic vein. After 15 min of morphine pretreatment, dogs received 100 mg/kg α-chloralose (Sigma Chemical Co., St. Louis, MO) through the same route. Chloralose was prepared by dissolving 3 g of powder in 300 ml of hot (70° C) 0.9% normal saline with stirring and cooling before the injection of a final concentration of 10 mg/ml. Anesthesia was maintained with continuous infusion (Harvard Apparatus, Southnatic, MA) at a rate of 15-20 mg/kg/hr, to preserve the autonomic control and minimize the infused volume without compromising the depth of anesthesia nor causing animal discomfort (Silverman and Muir III 1993).

Animals were intubated with a cuffed-tracheal tube (I.D.7.5 mm, Mallinckrodt Inc., Glens Falls, NY) that was inflated up to 16-20 mmHg to insure sufficient
ventilation. The artificial ventilator (Model 613, Harvard Apparatus Co. Inc., Mills, MA) was connected to the tracheal tube by a custom-made T-shaped glass tube connector with several openings to permit sampling the end-tidal PCO$_2$ and O$_2$%, airway pressure recording, and to introduce a catheter through the tracheal tube to record bronchial pressure. A tidal volume ($V_t$) of 15-20 ml/kg and respiratory rate of 15 breaths/min were kept constant during the whole period of the experiments with attention paid to maintain the baseline blood gases PCO$_2$, PO$_2$, and pH within the normal limits (35-45, above 90 mmHg, and 7.3-7.4, respectively). In addition, the body temperature was controlled during the surgical preparations and experiments ($37.0^\circ \pm 0.5^\circ$ C) by a water-heated blanket.

### 2.2.3 Instrumentations:

Two 7-French polyethylene catheters (Cordis Corporation, Miami, FL) were placed into the femoral artery and vein for blood gases analysis and drugs infusion, respectively. The jugular vein was exposed to insert a 5-F Swan-Ganz balloon-tipped double lumen catheter (Pediatric Baxter 746-5F Baxter-Edwards Healthcare Corporation, Irvine, CA) connected to a fluid filled transducer (T12 AD-R Viggo-Spectramed, Oxnard, CA) to measure the pulmonary arterial pressure (distal opening) and to inject (proximal opening) saline for cardiac output measurement (see below). The carotid artery was catheterized to measure left ventricular pressure by a Millar catheter (SPC-350-5F, Millar Instruments Inc., Houston, TX) connected to a Millar transducer. A 4-F balloon-tipped catheter (116F4, Edwards Lifesciences LLC, Irvine, CA) was threaded through the T-shaped glass tube to end at least in the second generation of the bronchi to record bronchial pressure. The opening of the glass tube was sealed around the catheter to assure
an air-tight connection and the external end of the catheter was connected to a fluid filled transducer. To confirm the location of catheters, all aforementioned procedures were performed under fluoroscopic monitoring (Siemens C-arm R.S.T. Sire Mobile 3U, Germany). Lead II ECG electrodes were placed on the left and right thoracic and pelvic limbs to monitor and record the heart rate (HR).

2.2.4 Measured and Calculated Parameters:

The cuff of the tracheal tube was inflated with a constant pressure of (mean ± S.E) 18 ± 0.6 mmHg at the baseline. Output from the cuff was connected to an electronic transducer (Biopac System Inc., Santa Barbra, CA) to record and monitor tracheal pressure (Tp). Airways pressure (Paw) was measured inside the tracheal tube via an opening in the T-shaped connector tube and recorded on the biopac system. Bronchial pressure (Brp), pulmonary artery pressure (Pap), and left ventricular end-diastolic pressure (LVEDP) were monitored by an E for M machine (VR-12 Stimultrace, Honeywell Inc.) and then recorded on the biopac system. The balloon-tipped catheter of Brp was inflated to give a constant pressure of (mean ± S.E) 28 ± 2.4 mmHg at the baseline. Cardiac output (CO) was measured by the thermodilution technique (Ganz et al., 1971; and Hoeper et al., 1999). In this technique, 5 cc of normal saline at a temperature of 20-23º C was injected into the right atrium via the Swan-Ganz catheter (proximal opening) and the blood temperature in the pulmonary trunk was recorded (distal thermistor) to compute the drop in temperature and calculate CO (Edwards COM-2 Cardiac Output Computer, Baxter Healthcare Corporation, Irvine, CA). All outputs from transducers and ECG electrodes were digitized at a frequency of 1 kHz and recorded for analysis on a 100 MP (MP 100 A-CE, Biopac System Inc., Santa Barbra,
CA) connected to a personal computer for digital storage. Heparinized blood samples were withdrawn from the femoral artery for pH and blood gases analysis (Gem Premier 3000, Instrumentation Laboratory, Lexington, MA). End-tidal gases (PCO₂ and O₂%) were sampled on-line via a second opening in the T-shaped tube and measured electronically (DATEX, 254 Airway Gas Monitor, Instrumentarium Corp., Helsinki, Finland). All equipment and transducers were calibrated before starting the experiments by using a sphygmomanometer to determine low (atmospheric pressure = 0 mmHg) and high (100 mmHg) pressure points.

Calculated parameters were derived from the recorded values after analyzing the last minute of each trace during every period. Pulmonary vascular resistance (PVR) was calculated as the difference between left ventricular end-diastolic and pulmonary artery pressures divided by the cardiac output; therefore, \( PVR = \frac{\text{LVEDP} - \text{Pap}}{\text{CO}} \) (mmHg/l/min).

To calculate pulmonary compliance (PC), a reciprocal of elastance or stiffness (the ability to recoil), the relationship between the volume and pressure difference was exploited, i.e. compliance = \( \frac{\text{volume}}{\Delta \text{Pressure}} = \frac{1}{\text{stiffness}} \) (Staub, 1998). As mentioned above, the tidal volume was fixed for each experiment (15-20 ml/kg) which was multiplied by the body weight to get the total tidal volume. The difference in airway pressure was calculated between each peak of inspiratory and expiratory strokes (\( \text{Paw}_i - \text{Paw}_e \)). Therefore, the \( \frac{\text{PC}}{\text{Paw}} = \frac{\text{V}_t}{\Delta \text{Paw}} \) (ml/mmHg) was obtained for each dog during different periods of an individual experiment.
2.2.5 Experimental Design and Protocol:

All eight dogs were subjected to the same protocol of stimulation and received the same treatments over synchronized periods of time (Table 2.1). After the procedures of anesthesia, intubation and instrumentations, a baseline period of 15 min was allowed for the preparation to equilibrate before recording the physiological parameters. During the baseline or control period, the tracheal and bronchial catheter balloons were inflated up to (mean ± S.E) 18 ± 0.6 and 28 ± 2.4 mmHg, respectively (Figures 2.1 and 2.2). It is very crucial to preset Tp and Brp in order to evaluate the influence and changes caused by the next stimulus.

The first respiratory stimulus was exposing each dog to a hypercapnic (5% CO₂ and 95% O₂) gas mixture for a period of 10 min to produce hypercarbia. To deliver the gas, a 30 liter non-elastic plastic breathing bag was filled with the mixed gases and attached to the respirator inlet without exerting any positive pressure. Fifteen minutes recovery period was permitted before starting the next stimulus. The second stimulus was the exposure to a hypoxic (10% O₂ and 90% N₂) gas mixture for a period of 10 min delivered by the same procedure as in hypercarbia. A second recovery period of 15 min was elapsed before the next stimulus. The third stimulus was activation of the parasympathetic autonomic control of the respiratory system. To achieve that, 0.5 mg/kg bethanechol at a concentration of 2 mg/ml (Sigma Chemical Co., St. Louis, MO) was injected intravenously, via the femoral vein, very slowly to minimize the effect on the heart rate. The effects of bethanechol were monitored for the 15 and 30 min periods after the injection to ensure continuous impact of the stimulus.
At the last minute of each stimulus or recovery period, heparinized blood samples were withdrawn from the femoral artery to obtain PCO₂, PO₂ and pH as well as on-line recording of end-tidal PCO₂ and O₂%. Cardiac output measurements were made after blood sampling and repeated, consecutively, 3 times to report the average. In addition, all of the other parameters were, simultaneously, displayed (Figures 2.1 and 2.2) and stored on a personal computer for further analysis.

2.2.6 Data Analysis:

Analyses were performed during the last 60 seconds of the baseline and after each stimulus or recovery period (Figure 2.1). All values represent the mean ± S.E.M of eight dogs for a particular parameter (Table 2.1). Statistical comparisons between each stimulus or recovery period and the baseline were sought by using Proc Mixed, a procedure in SAS. A longitudinal repeated measures analysis was used to examine the overall treatment effects over time as well as the pairwise comparisons between treatments or recovery periods. The Bonferroni correction for multiple comparisons was used to adjust for the number of tests being done. Therefore, the significance level (α) will be 0.05 divided by the number of treatments or recovery periods compared to the baseline, i.e. $\alpha = 0.05/7 = 0.007$. Thus, the significance level of $\alpha \leq 0.007$ was used to compare each stimulus or recovery period to the baseline. Generally, any p-value that is less than or equal to 0.01 is considered a significant difference (p-value $\leq 0.01$).
2.3 RESULTS:

2.3.1 The Effects of Hypercarbia:

Although dogs were exposed to a 10 fold (5%) higher than normal (0.5%) concentration of CO₂ for 10 min, most of the hemodynamic and respiratory changes were minimal.

The hemodynamic effects of high CO₂ include slight and nonsignificant (p>0.01) increases in HR, Pap, LVEDP, and no changes in CO from the baseline or other stimuli/recovery periods (Table 2.1 and Figures 2.3, 2.6, 2.8, and 2.9). PVR decreased insignificantly (p>0.01) from the baseline (Figure 2.9).

The respiratory effects of hypercarbia were more obvious compared to the baseline and recovery periods; however, they did not achieve statistical significance (p>0.01). These changes include a slight decrease in Tp and pulmonary compliance PC, which caused the subtle increase in Brp and Paw (Table 2.1 and Figures 2.3, 2.7, and 2.10). The changes in blood gases, pH, and end-tidal gases were all significant (p<0.01) compared to the baseline and other stimuli. The blood gas values were PCO₂ 50.7 ± 3.94, PO₂ 336.4 ± 73.04 mmHg, and pH 7.2 ± 0.02, whereas baseline values were PCO₂ 35.2 ± 1.63, PO₂ 93.8 ± 2.13 mmHg, and pH 7.4 ± 0.02. End-tidal gases were PCO₂ 61.1 ± 0.52 mmHg and O₂% 93.8 ± 0.37 during hypercarbia versus the baseline values of PCO₂ 36.0 ± 0.60 mmHg and O₂% 19.8 ± 0.16 (Table 2.1 and Figures 2.11 and 2.12)

2.3.2 The Effects of Hypoxia:

The hypoxic (10% O₂) stimulus effects were more pronounced on hemodynamic and respiratory parameters compared to hypercapnic stimulus effects (Figure 2.4).
The hemodynamic responses to low inspired concentration of O\textsubscript{2} include significant (p<0.01) increases in HR (141.1 ± 15.07 bpm), Pap (35.7 ± 1.70 mmHg), CO (3.4 ± 0.51 l/min), and PVR (5.5 ± 0.90 mmHg/l/min). The baseline values were as follow: HR (68.4 ± 3.36 bpm), Pap (14.5 ± 0.75 mmHg), CO (1.7 ± 0.18 l/min), and PVR (0.8 ± 0.20 mmHg/l/min). There was an increasing trend in LVEDP after hypoxia (19.2 ± 1.72 mmHg) compared to the baseline (13.7 ± 0.52 mmHg); however, this difference did not reach the statistical significance level (p=0.02). All these changes are depicted in Table 2.1 and Figures 2.6, 2.8, and 2.9.

The changes in the respiratory parameters in response to hypoxia were less severe than the changes in HR and other hemodynamics (Figure 2.4). The most noticeable reaction was the late increase in Tp from baseline (18.0 ± 0.57 mmHg) to (29.3 ± 5.18 mmHg) after hypoxia; nevertheless, this increase was not statistically significant versus the baseline (p=0.013) but was very significant versus Tp decrease after hypercarbia (P=0.009). Changes in Tp were observed in only 5 out of 8 dogs and occurred very late during the onset of hypoxia with an oscillatory pattern. The changes in Paw and Brp were less accentuated; although, there was a trend to increase (Table 2.1 and Figure 2.7) that did not reach statistical significance versus the baseline (p values=0.24 and 0.12, respectively). Figure 2.10 shows the decrease in PC from the baseline (159.4 ± 32.03 ml/mmHg) to (109.8 ± 14.84 ml/mmHg) after hypoxia without achieving the statistical significance (p=0.36). Blood gases and pH changes (Figure 2.11) were nonsignificant (p>0.01) compared to baseline and other stimuli; however, the decrease in end-tidal O\textsubscript{2}% (Figure 2.12) was significant (9.0 ± 0.27% vs. 19.8 ± 0.16%, p<0.01).
All the changes mentioned above did not persist after the termination of the hypoxic breathing. In fact, most of the significant responses returned to approximately the baseline values during the recovery period following the stimulus. Moreover, when the respiratory rate was increased from 15 to 25-30 breath/min, these changes were less appreciable, (hyperventilation effect in pilot experiments).

2.3.3 The Effects of 0.5 mg/kg Bethanechol:

Figure 2.5 and Table 2.1 display the hemodynamic and respiratory effects of 0.5 mg/kg bethanechol injected slowly via the femoral vein. As seen on the traces, using a parasympathomimetic drug to activate the parasympathetic limb was the most effective and sustained stimulus.

A few seconds (5-10 sec) after bethanechol injection, HR stopped for 1-3 sec then started with a lower rate than the baseline for 10 min. During the 10 min period after injection, severe bradycardia with escaping beats was the trait among all dogs. After 10-13 min of injection, HR increased (78.1 ± 14.93 bpm) compared to the baseline; however, this increase did not reach the significance level (p=0.4, Figure 2.6). Pap and LVEDP increased, significantly (p<0.0001), from the baseline (14.5 ± 0.75 and 13.7 ± 0.52 mmHg) to 26.0 ± 1.31 and 24.9 ± 2.51 mmHg after bethanechol, respectively (Figure 2.8). The increased Pap caused a significant (p=0.001) increase in PVR (3.7 ± 0.85 mmHg/l/min) from baseline (0.8 ± 0.20 mmHg/l/min), which was maintained for an extended period of time (Figure 2.9). On the other hand, CO did not change significantly (2.2 ± 0.41 vs. 1.7 ± 0.18 l/min, p=0.2) because HR did not change (Figure 2.9). It should be mentioned that the LVEDP increase diminished after 15 min of bethanechol injection, whereas elevated Pap lasted until the end of the experiment.
The responses of the respiratory parameters to this stimulus were very much augmented. Tp and Paw, but not Brp, were significantly increased after the stimulation of the vagal nerve by bethanechol injection (Figures 2.1 and 2.7). The simultaneous increases of both Tp and Paw from the baseline (18.0 ± 0.57 and 1.7 ± 0.29 mmHg) to 55.8 ± 3.05 and 3.3 ± 0.34 mmHg were very significant (p<0.0001 and p=0.001, respectively). Despite the continuous tracheal constriction, Paw decreased gradually until it reached the baseline level by the end of 15 min period after injection. The noticeable decrease in PC, from 159.4 ± 32.03 to 73.7 ± 13.88 ml/mmHg, after bethanechol was due to the synergistic increases in Pap and PVR (Figure 2.10), although this decrease seems not to be of a significance value (p=0.1). Figures 2.11 and 2.12 show that there were no significant changes (p>0.01) in blood and end-tidal gases after bethanechol; however, the decrease in pH from 7.4 ± 0.02 to 7.2 ± 0.03 was significant (p<0.001).

In summary, the most provocative effects on HR, Pap, PVR, and CO were after the exposure to hypoxia. Bethanechol was more effective in provoking tracheal constriction as well as increasing Paw, Pap, LVEDP, and PVR. None of these stimuli affect the PC significantly, in spite of the decremental tendency. Bronchi were not very responsive to any stimulus even with high tracheal constriction. The blood gases and pH changes were more sensitive to hypercapnic breathing, although pH decreased after bethanechol. It should be mentioned that bethanechol caused unavoidable and moderate parasympathetic side effects such as induced micturition, defecation or loose stool (diarrhea), increased salivation, and abdominal cramps.
2.4 DISCUSSION AND CONCLUSIONS:

The aim of these experiments was to find the strongest and most prolonged stimulus that gives the characters of tracheo-bronchoconstriction resembling those found in asthmatic patients including the hemodynamic and blood gases changes. To achieve that, three stimuli were tested in morphine/chloralose anesthetized dogs. These stimuli include the exposure to hypercapnia, followed by recovery period, then hypoxia, followed by second recovery period, and finally the stimulation of parasympathetic autonomic nervous system by the intravenous injection of 0.5 mg/kg bethanechol.

Hypercapnia did not, significantly, alter the hemodynamic or the respiratory parameters compared to baseline. The significant changes in blood gases, pH, and end-tidal gases were ascribed to the mixed percentages of gases, 5% CO₂ and 95% O₂. Hypoxia caused very severe tachycardia that was attributable to oxygen deprivation sensed by chemoreceptors and baroreceptors due to low PO₂. The tremendous elevation of HR caused significant increase in CO, which is a very rare incident in asthma. In addition, hypoxia caused enormous increases in Pap and PVR, which exist in very acute and congestive pulmonary diseases. The nonsignificant increase of Tp, after hypoxia, was the only positive effect that was observed in this research; yet, it is outweighed by the disadvantages mentioned above. Moreover, the effects elucidated by different gases were abolished by either termination of the stimulus (gas off) and by increasing the breathing rate per minute (hyperventilation). Bethanechol, on the other hand, produced sustained and significant increases in Tp, Paw, Pap, and LVEDP without affecting HR or CO. These changes were not susceptible to hyperventilation, by increasing breathing rate, or to the time factors. In spite of the conspicuous decrease in PC after hypoxia and
bethanechol, this reduction was not of a statistical significance. It is documented that bethanechol is not sensitive to acetylcholinesterase activity which prolongs its action (Brown and Taylor, 2001). Also bethanechol has no nicotinic (N) receptors activity which produces its effects in only muscarinic (M) receptors rather than affecting the neuromuscular junctions or ganglia. In addition, bethanechol has minimal effects on the cardiovascular system because it is relatively selective to M₁ and M₃ receptors. The dose of 0.5 mg/kg of bethanechol (i.v) was below the subcutaneous therapeutic dose of 1 mg/kg twice daily to treat urinary bladder atony in cats and dogs (Adams, 2001).

The advantages of bethanechol over the disadvantages promote the reliability of parasympathomimetics in inducing tracheo-bronchoconstriction that simulates asthma, which is demonstrated in the present study. Therefore, this conclusion leads to the acceptance of the hypothesis that bethanechol is effective in producing prolonged hemodynamic and respiratory changes more than hypercarbia or hypoxia in morphine/chloralose anesthetized dogs.

The effect of different gas inhalation on the respiratory system has been extensively studied; yet, it is the most controversial issue (Green and Widdicombe, 1996). In the literature, the contradictory debate over the effects of hypercapnia and hypoxia on the tracheo-bronchial responses is overwhelming (see Introduction). The majority of studies suggested that hypercapnia has no appreciable effects on tracheal motor tone or pulmonary resistance (Hirota et al., 2001; Lau et al., 1992; Mitchell and Vidruk, 1987; and Hilberman et al., 1976). Nonetheless, other investigators related the hypercapnic effects to exacerbation of other stimuli or conditions, such as inhalation of prostaglandin E₂ (Midorikawa et al., 1994), in asthmatics (Wesolowski, 1989), and the
rhythmic bronchoconstriction (Kondo et al., 2000 a & b). In contrast, D’Angelo et al., 2001, studied acute hypercapnia in human where they found that increasing PCO$_2$ from 20 to 65 mmHg caused bronchodilation in paralyzed and anesthetized (diazepam and isoflurane) subjects. Hypoxia also is one of the conflict points in the literature, although a considerable number of researchers concluded that low inspired O$_2$% caused noticeable bronchoconstriction without significant changes in pulmonary resistance. For example, Yu et al., 1984, in methacholine-induced bronchoconstriction in anesthetized cats found that hypoxia (4.5% O$_2$) significantly increased total diaphragmatic electromyographic (EMG) activity without significant changes in minute ventilation (VE) which indicates no changes in pulmonary compliance. Tam et al., 1985, reported that hypoxia (8 % O$_2$), in asthmatic humans, potentiated the bronchoconstrictive responsiveness as well as increased heart rate but the specific airways resistance did not increase. The latter results are consistent with some of the results in the present work, specially the effects on HR and PC. Hypoxia enhances the nonspecific bronchial reactivity due to the degranulation of mast cells and the consequences of histamine release which promotes bronchoconstriction. Hypoxia (13% O$_2$) enhanced, significantly, the effects of histamine and carbachol inhalations on the specific lung resistance (SRL) in conscious sheep (Ahmed and Marchette, 1985). The hypoxic (15% O$_2$) enhancement of bronchial responsiveness in awake sheep has been attributed to the stimulation of the carotid body chemoreceptors that influences the nervous control of the airway caliber (Denjean et al., 1991). In non-anesthetized newborn kittens, hypoxia (10% O$_2$) decreased minute ventilation with increased respiratory frequency and diaphragmic activity indicating either pulmonary bronchoconstriction or mechanical uncoupling of diaphragm and chest
wall (Rigatto et al., 1988). In stable asthmatic patients hypoxia, but not hyperoxia, exacerbated methacholine-induced bronchoconstriction (Dagg et al., 1997). The specific lower airways conductance (sGlaw) and expiratory reserve volume (ERV) were decreased in response to low oxygen (10% O₂) in dogs, indicating increased resistance and bronchoconstriction which were reversed by re-oxygenation but not by atropine (Watney et al., 1988). In contrast, the in situ left lower lobe study in canine lung has shown that varied degrees of hypoxia did not, significantly, affect bronchomotor tone or compliance (Coon et al., 1975). The bronchodilation effect of hypoxia (50, 28, or 0% O₂ + 5% CO₂) in porcine bronchial rings has been speculated to be the result of the opening of adenosine triphosphate-sensitive potassium channels (K⁺ATP) (Lindeman et al. 1994). Another argument for hypoxic (7% O₂) bronchodilation was deduced after visualizing the lung by high resolution computed tomography (HRCT) in anesthetized minipigs before, during and after hypoxic ventilation (Wetzel et al., 1992). The authors thought that the decreased resistance in upper airways and larynx would be applicable to the lung and lower airways. They reported an acute and reversible increase in small airways diameter more than those in large airways, 90 and 56%, respectively, although there was a slight increase in Paw from 18.5 to 18.9 Torr. To explain the conflicts between their findings and others’, they suggested methodological differences such as species differences, awake vs. anesthetized, and paralyzed vs. non-paralyzed subjects. In fact, pentobarbital anesthesia is known to prevent the release of acetylcholine from parasympathetic nerve endings and or to inhibit transmission along postganglionic parasympathetic nerves, which masks the vagal bronchoconstriction response (Jackson and Richards, 1977). In addition, the constriction in the lower airways is a rhythmic contraction in nature (Kondo
et al., 2000-b) and the imaging process could be during the bronchodi-latory phase rather than the constrictive phase. Other factors that could have influenced the bronchomotion are the tracheostomy and the forced positive end-expiratory pressure (PEEP) of 7 cmH₂O. The speculated rationale of hypoxic bronchodilation was due to the vasoconstriction in pulmonary veins which should be matching the ventilation/perfusion (V/Q) by relaxing the airways. Indeed, the contrary would be true because bronchoconstriction decreases the dead space and limits the local irritant effect of hypoxia to match the decrease in pulmonary blood flow. This bronchoconstriction is a well known physiological phenomenon that is experienced in high altitude environment where the decreased oxygen percentage causes chest tightness (airway obstruction), tachypnea (rapid, shallow breathing and decreased expiratory flow), and pulmonary vasoconstriction (Kryger et al., 1978).

The other methods of inducing bronchoconstriction in the previous works were by stimulating the vagal nerve either electrically or pharmacologically. The vagal electrical stimulation involves very weak sympathetic activation because it is difficult to isolate only one nerve limb and also it stops heart rate which affects other parameters (Green and Widdicombe, 1966; and Kondo et al., 2000- a& b). Depending on the intensity, duration, and frequency of the stimulation, the overwhelming release of acetylcholine would cause non-specific activation of muscarinic and nicotinic receptors including cardiovascular effects. The pharmacologic induction of bronchoconstriction can be produced by direct stimulation of muscarinic receptors on the smooth muscles via inhalation or injection of cholinergic drugs. Histamine inhalation is commonly used through aerosol delivery (Lunteren et al., 1984); however, it is deposited into large more than small airways,
which causes a non-uniform effect (Ruffin et al., 1978). The residual particles of histamine would irritate the mucosal layers of airways which in turn release more inflammatory mediators. In addition, histamine has many other side effects due to the lack of selectivity, which may, if injected, alter the response of airways because of the massive vasodilatation and edema that decrease lung compliance and increase resistance (Cardell et al., 1998). The injectable parasympathomimetics such as methacholine and carbachol are widely used to induce prolonged and consistent bronchoconstriction in paralyzed subjects to avoid nicotinic receptor activation (Breen et al., 1987; and Amirav et al., 2001). Acetylcholine has also been used in some studies; however, it should be infused over the whole period of experiment due to the degradation by acetylcholinesterase (Sterling et al., 1972; and Kondo et al., 2000-a). Bethanechol, on the other hand, has less effect on the nicotinic receptors with longer half life ($t_{1/2}$) than other cholinergics after bolus injection. It has been used in in vitro studies to constrict bronchial smooth muscle of rats (Szarek, 1989; and Szarek et al., 1997) and guinea pigs (Micheletti et al., 1987). To date, the present study was the first attempt to use bethanechol in intact dogs to induce acute respiratory and hemodynamic effects that emulate the tracheo-bronchial constriction seen in asthmatics. Most of the models that resembled acute asthma have exploited the intravenous infusion of methacholine (Rodriguez-Roisin et al., 1984; and Breen et al., 1987).

Many studies that have investigated the neural control of the airways reactivity concluded that tracheal contraction is the major factor in controlling airways caliber. However, Kondo et al., 1995, stated that “there were differences in contractions of extrathoracic trachea and the fifth-order bronchus in decerebrated and paralyzed dogs”.

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The cervical tracheal constriction was maintained during electrical vagal stimulation or after breathing hypercapnic gas, which was not the case in the fifth-order bronchus. In another study, Kondo et al., 2000-a, conducted experiments to investigate the role of cholinergic neural transmission on the airway resistance in decerebrated and paralyzed dogs. The effects of acute hypercapnia (100% CO$_2$), vagal stimulation before and after atropine (i.v) and the intravenous injection of acetylcholine were studied. They found that during acute hypercapnia airway resistance (Raw), represented by peak airway pressure (Paw), and tracheal tension (Ttr) increased significantly. However, electrical stimulation of the vagal nerve caused sustained increase in Ttr throughout the stimulation period while increase in Paw was not maintained. With acetylcholine, Ttr increased and decreased promptly while Paw lasted at high level only for few minutes. It was concluded that the bronchus is more sensitive to vagal stimulation and susceptible to the antagonism of acetylcholine than the trachea, which shows that contractile profiles of the 5th–order bronchus were reflected in airway resistance.

The preparation in the previous work of Kondo and his collaborators is different in many aspects from the methodology followed in the present study. First, removing the mid-brain, decerebration, affected the integrity of the central control on baro- and chemoreceptors that results in a dramatic reduction in arterial blood pressure and increased hypersensitivity to hypercapnia (Iscoe and Fisher, 1995). Moreover, paralyzing the skeletal muscle by pancuronium would affect the intercostal and diaphragm muscles, which alters the overall breathing pattern and airway compliance and resistance. Second, maintaining the intrathoracic negative pressure as near as normal and preserving the tracheal innervation are crucial steps in experiments that simulate the physiological
homeostasis, which would not be applicable with tracheostomy. Third, the tracheal pressure in the previous study was measured inside the tracheal tube and was predicted from the inspiratory flow and static pulmonary compliance. In the present experiments, tracheal pressure ($T_p$) was directly recorded from a cuff encapsulating the tracheal tube, which reflects precisely the changes in the tracheal diameter. Airways or intratracheal pressure ($P_{aw}$) in the present study was measured continuously via an opening in the T-shaped glass connector attached to the endotracheal tube (see Materials and Methods). The tidal volume and respiratory rate were preset and kept fixed during the baseline and the total period of the experiment, assuring that $PCO_2$, $PO_2$, and pH were within the normal levels. This protocol is an important element in the calculation of pulmonary compliance where a fixed and known tidal volume is divided by the difference between inspiratory and expiratory airway pressures (Amdur and Mead, 1958). Fourth, the choice of the anesthetic, chloralose, was based on the previous work conducted by Jackson and Richard, 1977, where they compared the effects of pentobarbitone and chloralose on the vagal component of bronchoconstriction produced by histamine aerosol. They showed, in vivo and in vitro, that chloralose, but not pentobarbitone, maintained the vagal reflex induced by either electrical stimulation or by histamine inhalation. The use of acetylcholine in the previous study had a very brief effect due to its short half life and metabolism by acetylcholinesterase, which is not the case for bethanechol (Adams, 2001; and Brown and Taylor, 2001). The fifth difference between the present study and the one done by Kondo and his colleagues, is the concentration of $CO_2$, where they used 20 times (100%) the percentage used in the present work (5%). Thus, in their results, the arterial $PCO_2$ increased from 36.1 to 79.7 Torr and the pH decreased from 7.36 to 7.11, which are
different from the correspondent values in this study. The calculated airway resistance
was derived from the peak pressure (Paw), which is consistent with the method used in
the present work to calculate pulmonary compliance utilizing the fixed tidal volume. In
addition, their results showed that hypercapnia increased tracheal tension while it was
less effective on airway resistance. This effect was not warranted in the present results
due to the differences in CO\textsubscript{2} concentrations and the hyperreactivity of airways to acute
hypercapnia and hypoxia (Iscoe and Fisher, 1995). The insignificant decrease in PC or
increased resistance in both studies would be attributed to the rhythmic constriction of
bronchi rather than tonic constriction as in the trachea (Kondo et al., 1995 and 2000-b).
Another explanation of the slight decrease in compliance is that the peripheral airways
are not well innervated by the parasympathetic nervous system (Jackson and Richard,
1977). Alternatively, the calculated compliance in the present study is the “dynamic
compliance” which represents lung stiffness because no attempts have been made to
measure intrapleural or esophageal pressures and the rate of airflow to determine the lung
resistance (Staub, 1998). Delivering a fixed volume and measuring the changes in
airways pressure is relatively less sensitive to changes in the lung resistance to airflow
than to changes in the lung stiffness (Widdicombe, 1966). Increased lung stiffness or
decreased compliance is one of the major determinants of pulmonary vascular
congestion, edema, and changes in alveolar-air interface but does not reflect a
bronchoconstriction, which should be measured directly as a change in airway caliber.

The disagreement between the present results and other previous models is due to
different experimental procedures such as anesthetics, gases concentrations, exposure
time, different cholinergic drugs, route of administration, and species. The interpretations
of the model discussed above should be extrapolated with caution to the clinical aspect of asthma, since there are many factors involved in the pathogenicity of the disease. Nevertheless, most of the clinical symptoms accompanying acute asthma could be demonstrated by activating the parasympathetic nervous control of the respiratory system. A single bolus intravenous injection of bethanechol should suffice prolonged and persistent bronchoconstriction and hemodynamic changes. In general, the limitations mentioned above do not hinder from using the bethanechol-induced tracheo-bronchoconstriction model to test new therapeutic agents used in asthma.
Figure 2.1: Traces of the Baseline Recordings (2 sec/division):
From top to bottom, lead II ECG, tracheal pressure Tp, airways pressure inside the tracheal tube Paw, bronchial pressure Brp, pulmonary pressure Pap, and left ventricular pressure LVP.
Figure 2.2: Traces of the Baseline Recordings (2 min/division):
From top to bottom, lead II ECG, tracheal pressure Tp, airways pressure inside the tracheal tube Paw, bronchial pressure Brp, pulmonary pressure Pap, and left ventricular pressure LVP.
Figure 2.3: Recordings after Exposure to Hypercarbia (5% CO2 + 95% O2):
Notice the slight and late increases in HR and Brp with no other changes.
Figure 2.4: Recordings after Exposure to Hypoxia (10% $O_2$ + 90% $N_2$):
Notice the high increases in HR, Pap and LVP. Also there were moderate increases in Tp, Paw with very slight changes in Brp.
Figure 2.5: Recordings after Treatment with 0.5 mg/kg Bethanechol:
Notice that heart stopped for seconds and then HR decreased for 10 min then went back to or slightly above normal. Also there were high increases in Tp, Paw, Pap, and moderate increase in LVP with very slight or no changes in Brp.
<table>
<thead>
<tr>
<th>Stimulus/Recovery</th>
<th>Heart Rate (bpm)</th>
<th>Tp (mmHg)</th>
<th>Paw (mmHg)</th>
<th>Erp (mmHg)</th>
<th>Pap (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>Cardiac Output (l/min)</th>
<th>Pul. Vascular Resistance</th>
<th>LVEDp-Pap/CO</th>
<th>Pul. Compliance Vd/ΔPaw</th>
<th>Blood Gases</th>
<th>PCO₂</th>
<th>PO₂</th>
<th>pH</th>
<th>End Tidal PCO₂</th>
<th>O₂%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>68.4 ± 3.36</td>
<td>18.0 ± 0.57</td>
<td>1.7 ± 0.29</td>
<td>28.4 ± 2.38</td>
<td>14.5 ± 0.75</td>
<td>13.7 ± 0.52</td>
<td>1.7 ± 0.18</td>
<td>0.8 ± 0.20</td>
<td>159.4 ± 32.03</td>
<td>35.2 ± 1.63</td>
<td>93.8 ± 0.13</td>
<td>7.4 ± 0.02</td>
<td>36.0 ± 0.60</td>
<td>19.8 ± 0.16</td>
<td></td>
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</tr>
<tr>
<td>High CO₂ (5%)</td>
<td>72.5 ± 3.75</td>
<td>17.4 ± 1.08</td>
<td>1.8 ± 0.28</td>
<td>31.4 ± 2.75</td>
<td>15.1 ± 0.77</td>
<td>15.2 ± 0.75</td>
<td>1.7 ± 0.20</td>
<td>0.6 ± 0.21</td>
<td>154.0 ± 30.78</td>
<td>30.7 ± 3.94</td>
<td>336.4 ± 0.13</td>
<td>7.2 ± 0.02</td>
<td>61.1 ± 0.62</td>
<td>93.8 ± 0.37</td>
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</tr>
<tr>
<td>15 min</td>
<td>72.7 ± 4.96</td>
<td>18.4 ± 0.68</td>
<td>1.8 ± 0.33</td>
<td>34.9 ± 2.67</td>
<td>15.8 ± 1.02</td>
<td>15.2 ± 0.85</td>
<td>1.7 ± 0.20</td>
<td>1.2 ± 0.25</td>
<td>201.8 ± 77.97</td>
<td>36.7 ± 2.65</td>
<td>95.0 ± 0.24</td>
<td>7.4 ± 0.20</td>
<td>39.8 ± 0.13</td>
<td>19.5 ± 0.27</td>
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</tr>
<tr>
<td>Low O₂ (10%)</td>
<td>141.1 ± 15.07</td>
<td>29.3 ± 5.18</td>
<td>2.2 ± 0.28</td>
<td>34.6 ± 2.81</td>
<td>35.7 ± 1.70</td>
<td>19.2 ± 1.72</td>
<td>3.4 ± 0.51</td>
<td>5.5 ± 0.90</td>
<td>109.8 ± 14.84</td>
<td>33.3 ± 3.79</td>
<td>33.8 ± 0.13</td>
<td>7.4 ± 0.20</td>
<td>39.5 ± 0.13</td>
<td>9.0 ± 0.27</td>
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<tr>
<td>15 min</td>
<td>73.8 ± 4.26</td>
<td>18.7 ± 0.85</td>
<td>1.7 ± 0.31</td>
<td>34.4 ± 2.87</td>
<td>17.3 ± 0.63</td>
<td>16.8 ± 0.52</td>
<td>1.7 ± 0.17</td>
<td>0.8 ± 0.19</td>
<td>168.3 ± 39.66</td>
<td>34.2 ± 12.20</td>
<td>86.9 ± 0.16</td>
<td>7.4 ± 0.20</td>
<td>37.9 ± 0.21</td>
<td>19.3 ± 0.16</td>
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<tr>
<td>0.5 mg/kg Bethanechol</td>
<td>78.1 ± 14.93</td>
<td>55.8 ± 0.34</td>
<td>3.3 ± 2.91</td>
<td>34.7 ± 1.31</td>
<td>26.0 ± 2.51</td>
<td>24.9 ± 0.41</td>
<td>2.2 ± 0.41</td>
<td>3.7 ± 0.85</td>
<td>73.7 ± 13.88</td>
<td>39.6 ± 2.95</td>
<td>58.5 ± 0.30</td>
<td>7.2 ± 0.03</td>
<td>37.6 ± 2.10</td>
<td>19.6 ± 0.18</td>
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<tr>
<td>15 min</td>
<td>94.4 ± 4.40</td>
<td>60.8 ± 4.83</td>
<td>2.1 ± 0.30</td>
<td>34.7 ± 2.92</td>
<td>21.3 ± 4.68</td>
<td>14.7 ± 1.12</td>
<td>2.1 ± 0.22</td>
<td>3.4 ± 0.63</td>
<td>159.0 ± 23.35</td>
<td>35.2 ± 2.35</td>
<td>75.5 ± 0.41</td>
<td>7.3 ± 0.20</td>
<td>38.4 ± 1.33</td>
<td>19.4 ± 0.18</td>
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<tr>
<td>30 min</td>
<td>88.3 ± 5.01</td>
<td>55.5 ± 3.12</td>
<td>1.9 ± 0.33</td>
<td>34.6 ± 2.08</td>
<td>20.8 ± 1.83</td>
<td>18.3 ± 2.00</td>
<td>2.0 ± 0.25</td>
<td>3.7 ± 0.74</td>
<td>145.2 ± 31.68</td>
<td>31.7 ± 2.39</td>
<td>81.4 ± 0.03</td>
<td>7.3 ± 0.18</td>
<td>36.3 ± 1.18</td>
<td>19.4 ± 0.18</td>
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</tbody>
</table>

Table 2.1: Changes in Respiratory and Hemodynamic Parameters in Response to Different Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol):
Mean ± SEM of 8 dogs. For statistical significance values see graphs.
Figure 2.6: Heart Rate Changes in Response to Different Respiratory Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol): Mean ± SEM of 8 dogs. (*) indicates the significant statistical difference in HR after only hypoxia compared to the baseline and other stimuli/recovery times (P-value <0.0001).
Figure 2.7: Tracheal, Bronchial and Airways Pressures Changes in Response to Different Respiratory Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol):
Mean ± SEM of 8 dogs. (*) indicates the statistical significant difference in Tp after bethanechol and 15 & 30 min recovery periods compared to the baseline and other stimuli (P-value <0.0001). (‡) indicates the statistical significant difference in Paw after only bethanechol compared to the baseline and other stimuli (P-value =0.001). No significant changes in Brp between the treatments.
Figure 2.8: Pulmonary artery and Left Ventricular End-diastolic Pressures Changes in Response to Different Respiratory Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol):
Mean ± SEM of 8 dogs. (*) indicates the statistical significant difference in Pap after hypoxia, bethanechol, and 15 & 30 min recovery periods compared to the baseline and other stimuli (P-value <0.0001). (‡) indicates the statistical significant difference in LVEDP after only bethanechol compared to the baseline and other stimuli (P-value <0.0001).
Figure 2.9: Cardiac Output and Pulmonary Vascular Resistance Changes in Response to Different Respiratory Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol):
Mean ± SEM of 8 dogs. (*) indicates the statistical significant difference in C.O after only hypoxia compared to the baseline and other stimuli (P-value =0.001). (‡) indicates the statistical significant difference in P.V.R. after hypoxia, bethanechol, and 15 & 30 min recovery periods compared to the baseline and other stimuli (P-value =0.001).
Figure 2.10: Pulmonary Compliance Changes in Response to Different Respiratory Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol):
Mean ± SEM of 8 dogs. Pulmonary compliance was calculated by dividing tidal volume (Vt) by the difference in airways pressure -inside the tracheal tube- (ΔPaw) between inspiration and expiration. No statistical significant difference was found between the treatments.
Figure 2.11: Blood Gases Partial Pressures of CO₂ & O₂ and pH Changes in Response to Different Respiratory Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol):
Mean ± SEM of 8 dogs. (*) indicates the statistical significant difference in PCO₂ after only hypercarbia compared to the baseline and other stimuli (P-value = 0.001). (‡) indicates the statistical significant difference in PO₂ after only hypercarbia compared to the baseline and other stimuli (P-value < 0.001). (§) indicates statistical significant difference in pH after hypercarbia, bethanechol and 15 min recovery time after bethanechol compared to the baseline and other stimuli (P-value = 0.001).
Figure 2.12: End-tidal Partial Pressure of CO$_2$ and O$_2$% Changes in Response to Different Respiratory Stimuli (Hypercarbia, Hypoxia, and 0.5 mg/kg Bethanechol):
Mean ± SEM of 8 dogs. (*) indicates the statistical significant difference in PCO$_2$ after only hypercarbia compared to the baseline and other stimuli (P-value >0.001). (‡) indicates the statistical significant difference in O$_2$% after hypercarbia and hypoxia compared to the baseline and other stimuli (P-value <0.001).
2.5 LITERATURE CITED:


3.1 INTRODUCTION:

The search for drugs that efficiently relieve bronchoconstriction with few side effects in asthmatic patients continues. It is believed that there is a strong impact of the autonomic nervous system on the pathophysiology mechanisms of tracheo-bronchial reactivity (Barnes, 1995). In the normal state, there is a balance between constrictor mechanism, which is mediated by parasympathetic, and inhibitory bronchodilating action of sympathetic. This balance may be disturbed by many factors that induce the release of endogenous bronchoconstrictors such as histamine from mast cells which in turn constrict airway smooth muscle and increase muscarinic receptor sensitivity to acetylcholine (Barger and Evans, 1991). As discussed in Chapters 1 and 2, many experimental models have been proposed to simulate the bronchoconstriction that is caused by an abnormal imbalance in the autonomic control of airways and perturbing substances that cause inflammatory responses in smooth muscle and mucous glands. Different approaches and protocols in animals and humans were designed to induce changes in airways caliber by electrical stimulation of nerves or by pharmacological agents that activate or inhibit autonomic receptors. Many studies have been done to understand the autonomic control
of respiratory system by exploiting agonist and antagonist agents of both adrenergic and cholinergic systems. Some of these studies were carried out in different species *in vitro* (Filbert et al., 1992; Barnes et al., 1983; and Akhtar et al., 1999), *in situ* (Leff et al., 1983), and *in vivo* (Cabezas et al., 1971; Hahn et al., 1976; Woolcock et al., 1969; Stepanek, 1978; and Weinmann et al., 1985). There is agreement among previous works that there is a trend of heterogeneity in the distribution of receptors along the respiratory tract with high density of cholinergic receptors in large airways smooth muscle, while adrenergic receptors are abundant in small and distal bronchioles (Barnes et al., 1983). Furthermore, substantial evidence supports the universal thought that parasympathetic innervation plays a major role in controlling airways caliber (Widdicombe, 1985).

Therefore, therapeutic strategies of bronchodilation stem from studies of animal models that explored autonomic innervation of the tracheo-pulmonary tree in an attempt to understand the mechanism of bronchoconstrictive diseases such as asthma and chronic obstructive pulmonary disease (COPD).

Three classical types of bronchodilators were introduced to clinical practice for the treatment of bronchospasm that accompanied asthma and COPD: antimuscarinics, β₂-adrenoceptors agonists, and phosphodiesterase (PDE) inhibitors (Karpel et al., 1994). In animals, application of these agents was extrapolated from the treatment regimens of asthmatic humans despite the intra-species variations in respect to physiological and anatomical properties (Boothe and McKiernan, 1992). In addition, several studies evaluated the therapeutic benefits as well as side effects of those bronchodilators; however, they were confined, primarily, to effects on the respiratory system. Nonetheless, these agents cause other unwanted effects such as stimulation of the central nervous
system (tremors, excitation, and seizures), gastrointestinal upset (nausea and vomiting), and cardiac stimulation. Most importantly, changes occur in the homeostasis of cardiovascular system, either favorable or undesired, that are less defined in the previous works. Other than alterations in the heart rate and blood pressure, few investigators have conducted studies that were dedicated to hemodynamic effects of bronchodilators such as inotropic influence of β-adrenoceptors agonists and aminophylline (Yabuuchi et al., 1977; Rodriguez-Roisin et al., 1984; and Matthay, 1987). Moreover, induced bronchoconstriction by several means to test drugs efficacy have appreciable impact on how these agents may correct this abnormality in well tolerated doses with fewer side effects. Cholinergic-induced bronchoconstriction such as by methacholine inhalation or injection is widely used to challenge airway smooth muscle in order to assess the bronchoprotective action of β2 agonists (Woude et al., 2001) and aminophylline (Hirota et al., 2001). However, methacholine has some nicotinic receptor activity which may alter both cholinergic and adrenergic neurotransmissions as well as cardiovascular parameters besides the fact that it is moderately susceptible to acetylcholinesterase degradation (see Chapter 2). Another issue in evaluating bronchoactive drugs in in vivo models is the choice of anesthetics, since some agents, such as pentobarbital, are known to mask vagal bronchoconstriction due to acetylcholine release inhibition (Jackson and Richards, 1997) and may change hemodynamic parameters as well. Routes of administration and dosages protocols can also influence the potency and duration of bronchodilators in experiments designed to be extrapolated to clinical practice. While it is important to deliver a drug directly to blood stream in order to circumvent first-pass metabolism by liver after absorption, usually bronchodilators, in aerosolized form, are given by inhalation from a
nebulizer to directly distributed to airways receptors. Nevertheless, in studies intended to investigate bronchodilator effects other than tracheo-pulmonary such as hemodynamic and unwanted side effects, intravenous injection is preferable because of uniformed and equal distribution to different targets.

In humans, several clinical trials have been conducted to compare the therapeutic values of different types of bronchodilators as an individual drug or multi drugs as a combination (McCrory and Brown, 2002; Friedman, 1997; and Mizuno, 1990). The purpose of different drugs combination, in low doses of each, is to overcome intolerable side effects (Wolfe et al., 1978) and to provide more efficient bronchodilation (Grootendorst et al., 2003; and Lu, 1997). In veterinary medicine, drug combinations are not commonly prescribed, unless there is refractoriness to monotherapy as the case with β₂ agonists because of receptors desensitization (Tobias et al., 1990); yet doses are still arbitrary and extended from human application (Boothe and McKiernan, 1992). Recently, there is a growing interest in developing more selective antimuscarinic drugs that are useful in the treatment of COPD as a single drug or combined with β₂ agonists and aminophylline in the relief of asthmatic exacerbations (Hansel and Barnes, 2002; Campbell, 2000; Witek, 1999; Garrett, 1997; Rubin and Abers, 1996; and Chapman, 1991 and 1990). These selective M₃ and M₁ antagonists were developed because of tachycardia that accompanied non-selective anticholinergics due to cardiac M₂ block, though high doses of the selective agents might not dissociate from M₂ very quickly, giving similar side effects. Therefore, it is very crucial to examine the cardiovascular effects of anticholinergics used in asthma and COPD, since little is known about their hemodynamic changes, especially alteration of pulmonary vascular resistance. The non-
selective antimuscarinics such as atropine and ipratropium produce bronchodilation in COPD patients due to blockade of M₃ receptors on airways smooth muscle. However, they also accelerate heart rate by blocking cardiac M₂ and, in low doses, they may potentiate mild bronchoconstriction by inhibiting the autoregulatory presynaptic M₂ receptors that control acetylcholine release (Abad Santos et al., 2003). Similarly, non-selective \( \beta \)-adrenoceptors agonists such as isoproterenol and high doses of PDE inhibitors such as aminophylline cause bronchodilation as well as increase heart rate as a consequence of increased cAMP (Adams, 1984; and Heaslip et al., 1991).

There is a real need for specific studies that compare not only respiratory but also hemodynamic effects of different bronchodilators on synchronized and controlled experimental settings in animals with few or no surgical interventions. More importantly, provoking a bronchoreaction should be achieved in a manner that simulates the pathophysiological state of prolonged and symmetrical bronchoconstriction, which usually can be accomplished pharmacologically. Preserving normal autonomic control of the cardio-pulmonary system is a major determinant of how the effects of drugs would be interpreted, since many anesthetic procedures may mask these changes (see above). Moreover, designing dosages and route of administration should be determined to allow normal therapeutic range of plasma concentrations which brings about the desired level of drug effect.

3.1.1 Objectives and Hypothesis:

Henceforth, the purpose of the experiments in this chapter is to compare the respiratory and hemodynamic effects of three major bronchodilators on the same standardized model of bethanechol-induced bronchospasm in anesthetized dogs. A
specific dosing protocol is followed in order to find the optimal bronchodilating doses with minimal alterations in cardiovascular system. Although \( \beta_2 \) agonists and PDE inhibitors are widely used in the treatment of asthma and COPD, their ability to oppose parasympathomimetic-induced bronchoconstriction is questionable. Therefore, the hypothesis to be tested is that atropine relieves bethanechol-induced tracheobronchoconstriction in morphine/chloralose anesthetized dogs and it is more effective than terbutaline or aminophylline with some favorable cardiovascular effects. Furthermore, this work will reconfirm the heterogeneity of autonomic receptors distribution on large versus small airways in the intact dog. In addition, the outcome of this study could be of significant benefit to clinical practice, since there is a trend in considering selective antimuscarinics in COPD and asthma such as long acting tiotropium and short acting oxitropium (Hansel and Barnes, 2002; and Disse, 2001).
3.2 MATERIALS AND METHODS:

3.2.1 Animals:

The experiments were approved by the Institutional Laboratory Animal Care and Use Committee (ILACUC) of The Ohio State University. Sixteen, young, mature and healthy beagle dogs of either sex weighing between 7.3 to 15.5 kg (mean ± S.D 9.94 ± 2.9 kg) were used in four groups (see experimental design protocol below). The animals were checked before the experiments to eliminate the possibility of preexisting cardiovascular or pulmonary abnormalities.

3.2.2 Anesthesia, Intubation, Ventilation, and Instrumentations:

Same anesthetic procedure as in Chapter 2 was followed in these experiments by using morphine/chloralose protocol. Tracheal intubation was performed in the same pattern of the previous work in which the cuff pressure was kept constant (18-20 mmHg) starting from the baseline and during each dose/time periods in all of the experiments. The tidal volume and respiratory rate were kept constant (15-20 ml/kg and 15-17 breath/min, respectively) in order to monitor changes in the pulmonary airways pressure (Paw) that is recorded instantaneously. Bronchial pressure (Brp) was recorded via a balloon-tipped catheter inserted through the tracheal tube to, at least, the second generation bronchus at where the balloon was inflated to a fixed pressure of 28-34 mmHg. A Swan-Ganz catheter was inserted through the jugular vein to measure the pulmonary arterial pressure (Pap) and cardiac output (CO) at the same time. The left carotid artery was catheterized to monitor left ventricular pressure by Millar catheter in order to measure end-diastolic pressure (LVEDP).
Blood samples, for gases and pH analyses, were obtained from the femoral artery and drugs were infused via the femoral vein. Heart rate (HR) was recorded from lead II electrodes placed on the left and right thoracic and pelvic limbs.

3.2.3 Drugs Preparation:

The clinical form of intravenous injectable aminophylline (25 mg/ml) was made by Abbot Laboratories, North Chicago, IL. Also injectable atropine (0.4 mg/ml) was used as formulated for the clinical practice by American Pharmaceutical Partners, Inc., Los Angeles, CA. Terbutaline was prepared the day of the experiments by dissolving 20 mg of the powder form (Sigma, St. Louis, MO) in 200 ml of normal saline to reach a concentration of 0.1 mg/ml.

3.2.4 Experimental Design, Protocol, and Measurements:

Sixteen animals were divided into four groups: aminophylline, atropine, terbutaline, and control or vehicle. Each group, consisting of four animals, received either four cumulative doses of one bronchodilator or saline with 10 min intervals between each dose. After recording the baseline for 15 min, bronchoconstriction was induced by intravenous bolus injection of 0.5 mg/kg bethanechol and 10 min period of time was allowed for equilibrium. Aminophylline was intravenously injected in cumulative doses of 2.5, 5, 10, and 20 mg/kg with 10 min intervals between doses and then its effects were monitored 10, 30, and 60 min after the last dose. The same protocol was followed for atropine in doses of 0.02, 0.04, 0.08, and 0.16 mg/kg and for terbutaline in doses of 0.002, 0.004, 0.008, and 0.016 mg/kg. These regimens represent the subclinical, clinical, two times, and four times the clinical bronchodilatory doses of a particular drug in man. Traces of ECG, tracheal (Tp), airways (Paw), bronchial (Brp), pulmonary arterial (Pap),
and left ventricular (LVP) pressures as well as cardiac output (CO) were monitored and recorded continuously. Ten minutes of each dose/time period was recorded for later analysis and calculation of left ventricular end-diastolic pressure (LVEDP), pulmonary vascular resistance (PVR), and pulmonary compliance (PC) (see Chapter 2 for further information of calculations). Blood and end-tidal gases were analyzed and recorded at the end of each dose/time period.

3.2.5 Data Analyses:

Analyses of the measurements were performed during the last 60 seconds of all the traces in each animal after each dose/time (Figures 3.1, 3.2, and 3.3). All values represent the mean ± S.E.M of four dogs in each group for a particular parameter (Tables 3.1, 3.2, and 3.3). Statistical comparison of the means was sought between the baseline and/or after bethanechol and each dose/time period. In addition, differences between treatment groups and the vehicle group were compared at each dose/time period for each parameter. By using Proc Mixed, a procedure in SAS, a longitudinal repeated measures analysis was used to examine the overall treatments effects as well as the pairwise comparisons between doses and the baseline and/or after bethanechol and between the treatment and the vehicle groups. Within any group, comparisons of the doses effects back to the baseline or after bethanechol were achieved by applying the Bonferroni correction for multiple comparisons; i.e. adjusting the level of significance $\alpha = (0.05/8) = 0.006$, where 8 is the number of comparisons made. Also, when comparing each group to the vehicle group at any dose/time, adjusted level of significance was used; i.e. $\alpha = (0.05/9) = 0.005$, where 9 is the number of treatments (dose/time). The peak effect and time to peak on the tracheal pressure of each bronchodilator group at the four doses were
compared using $\alpha = 0.05$; however, comparisons between the three bronchodilators were made by using $\alpha = (0.05/4) = 0.013$, where 4 is the number of doses compared. The latter significance level differs from the reminder of the parameters because the peak effect and time to peak were not measured at every dose/time period.
3.3 RESULTS:

Comparisons were made between each dose/time period and the baseline or after bethanechol within each group or between each bronchodilator group and the vehicle group by using \( p \leq 0.006 \). When comparing the peak effect on \( Tp \) of a particular bronchodilator at the four doses, \( p \leq 0.05 \) was used and the differences between the three bronchodilators were evaluated by using \( p \leq 0.013 \) because there were only 4 observations (see Materials and Methods).

3.3.1 The Hemodynamic Effects of Bronchodilators:

3.3.1.1 Heart Rate Changes:

Bethanechol, in any group, did not change the heart rate (HR) significantly \( (p>0.006) \). Heart rate accelerated significantly \( (p<0.001) \), in a dose-dependent fashion, after 0.04, 0.08, 0.16 mg/kg atropine (Figure 3.4 and Table 3.2). Baseline HR was 84.3±13.06 bpm then increased after the 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) doses to 165.4±5.53, 178.5±3.21, and 192.4±7.24 bpm, respectively. Although HR started to decelerate after the forth dose, it remained higher than the baseline and then decreased to 124.8±5.30 bpm after 60 min, which was significantly different from the baseline \( (p=0.003) \). No significant increases in HR after aminophylline, terbutaline, or vehicle at any dose or period of time, although with aminophylline, there was a tendency to increase that did not achieve the statistical significance \( (p>0.006) \). There was a significant difference \( (p<0.001) \) between the atropine and vehicle groups starting at 2\(^{nd}\) dose until the end of the experiment. Also, aminophylline differed significantly \( (p<0.001) \) from the vehicle starting at 4\(^{th}\) dose until 60 min after the injection. In contrast, terbutaline did not change HR at any dose or time period compared to the vehicle group \( (p>0.006) \).
3.3.1.2 Pulmonary Arterial Pressure Changes:

After bethanechol injection for 10 min, pulmonary arterial pressure (Pap) increased significantly (p<0.006) from the baseline (range of four groups) 12.2±0.87 - 15.0±0.66 to 21.8±1.34 - 24.0±0.69 mmHg after bethanechol in all groups (Figure 3.5 and Tables 3.1, 3.2, and 3.3). In the vehicle group only Pap remained, significantly (p<0.006), elevated and was higher than the baseline until the end of the experiment, although it declined nonsignificantly with the time. The first, second, and third doses of atropine (0.02, 0.04 and 0.08 mg/kg) decreased Pap significantly (p<0.006) to 15.7±1.25, 16.1±1.17, and 16.7±1.23 mmHg compared to after bethanechol (22.8±1.59 mmHg). Then Pap increased, nonsignificantly (p=0.011), after the fourth dose until the end of the experiment; however, this increase did not differ from the baseline (p>0.006). Aminophylline, at any dose/time, did not decrease Pap after the increase by bethanechol, despite the decreasing trend, which did not differ from baseline or after bethanechol (p>0.006). Terbutaline tended to decrease Pap after bethanechol at 1st, 2nd, and 3rd doses; however, this decrease did not reach the statistical significance (p values are: 0.012, 0.011, and 0.022). Moreover, Pap started to increase significantly (p<0.006) after the fourth dose of terbutaline to be higher than the baseline and remained high until 60 min after the injection where it was 20.4±2.90 mmHg. Among the three treatments, only atropine and terbutaline differed significantly (p<0.006) from vehicle at the first dose.

3.3.1.3 Left Ventricular End-Diastolic Pressure Changes:

In all of the four groups, left ventricular end-diastolic pressure (LVEDP) was elevated by bethanechol; however, this increase was significant (p<0.006) only in the vehicle and atropine groups (Figure 3.6 and Table 3.2). Atropine significantly (p<0.006)
decreased LVEDP from 18.9±1.28 mmHg after bethanechol to 14.0±1.04 mmHg after the first dose (0.02 mg/kg). This effect persisted during the periods of subsequent doses until the end of the experiment in which there were no differences between doses or the baseline values (p>0.006). Also, LVEDP declined significantly (p<0.006) compared to after bethanechol in the vehicle group, starting at 2nd dose until the end of the experiment, and in aminophylline group, at 10, 30, and 60 min (Table 3.1). No effects in the terbutaline group, at any dose/time, were noticed, although terbutaline seemed to increase LVEDP slightly without any significance (Table 3.3). There were no differences between any treatment group and the vehicle because the effect of bethanechol was not sustained for a longer period of time in all the groups, as can be deduced from the declined LVEDP in the vehicle group after 2nd dose.

3.3.1.4 Cardiac Output Changes:

Bethanechol did not affect the cardiac output (CO); thus no appreciable changes were seen after any treatment group at any dose/time (Figure 3.7 and Tables 3.1, 3.2, and 3.3). The slight increase in CO in some groups, such as vehicle and terbutaline, could be related to the volume changes after chloralose or saline injections.

3.3.1.5 Pulmonary Vascular Resistance Changes:

Pulmonary vascular resistance (PVR) increased after bethanechol from (range of the four groups) 1.3±0.46 - 2.1±0.89 to 2.4±0.86 - 4.7±3.21 mmHg/l/min, although this increase was not significant (p>0.006) (Figure 3.8 and Tables 3.1, 3.2, and 3.3). After the first dose, aminophylline showed some tendency to increase PVR insignificantly (p=0.56); atropine and terbutaline tended to decrease PVR insignificantly (p= 0.04 and 0.10, respectively) and no treatment group differed, significantly, from the vehicle group
(p>0.006). The lack of significant changes in PVR was due to the high variability among the animals, i.e. high standard error “SE”, and to the few changes in CO, LVED, and Pap.

3.3.2 The Respiratory Effects of Bronchodilators:

3.3.2.1 Tracheal Pressure Changes:

The tracheal pressure (Tp) was increased significantly (p<0.001) by bethanechol-induced constriction in all of the four groups (Figure 3.9). The baseline Tp was in the range of (four groups) 18.6±0.94 - 20.2±0.63 mmHg and increased after bethanechol to the range of 50.1±2.87 - 66.0±2.95 mmHg (Tables 3.1, 3.2, and 3.3). After the second and the third doses (0.04 and 0.08 mg/kg) of atropine, Tp decreased dramatically (p<0.001) to 27.1±4.69 and 18.4±1.68 mmHg, respectively. No further significant decreases occurred after third dose and no significant differences (p=0.9) were seen between doses of atropine. Starting at the second dose of atropine, Tp returned to baseline value; therefore, there were no significant differences (p>0.006) between the 2nd, 3rd, and 4th doses and the baseline until the end of the experiment. Aminophylline and terbutaline, at any dose, did not decrease Tp after it was elevated by bethanechol; therefore, Tp remained significantly (p<0.001) higher than the baseline. Among the three treatments, atropine after the second dose was the only bronchodilator that differed significantly (p<0.001) from the vehicle and the other two bronchodilators. The effects of aminophylline and terbutaline were very brief and they decreased Tp for a short period of time (Figures 3.1 and 3.3); therefore, their peak effects and times to peak were recorded and analyzed after each dose in order to be compared with those of atropine.
### 3.3.2.2 Peak Effects and Time to Peak on Tracheal Pressure:

The maximum effect and the time to achieve this effect on the tracheal pressure were compared among the three treatments (Figure 3.10 and Tables 3.1, 3.2, and 3.3). The second, third, and fourth doses of atropine (0.04, 0.08, and 0.16 mg/kg) decreased Tp significantly (p<0.001) compared to the first dose (0.02 mg/kg). However, there were no significant differences between the second, third, and fourth doses (p>0.05). In addition, atropine was more effective in decreasing Tp than aminophylline and terbutaline at those doses. Aminophylline, at the 4th dose (20 mg/kg), decreased Tp to 45.28±3.47 mmHg from 51.8±5.77 mmHg after bethanechol (12.58%). Atropine, at the 4th dose (0.16 mg/kg), decreased Tp to 17.45±1.60 mmHg from 50.1±2.87 mmHg after bethanechol (65.2%). Terbutaline, at the 4th dose (0.016mg/kg), decreased Tp to 35.03±7.85 mmHg from 66.0±2.95 mmHg after bethanechol (46.9%). Thus, among the three treatments, the peak effect of atropine (0.04, 0.08, and 0.16 mg/kg) on Tp was more pronounced than aminophylline and terbutaline (p<0.001). There was no significant difference between the peak effect of aminophylline and terbutaline (p>0.013). No significant differences have been found in the time to peak among the three treatments or between doses within any bronchodilator group.

### 3.3.2.3 Airway Pressure Changes:

Airway pressure (Paw) increased insignificantly in vehicle, aminophylline, and atropine groups (p values are: 0.027, 0.075, and 0.025, respectively) and significantly (p<0.001) in terbutaline group after bethanechol (Figure 3.11). The baseline Paw was (range of four groups) 1.1±0.39 - 2.1±0.22 mmHg, then increased to 2.2±0.31 - 4.3±0.47 mmHg after bethanechol (Tables 3.1, 3.2, and 3.3). Terbutaline, starting at the first dose
(0.002 mg/kg), decreased Paw significantly \((p<0.006)\) from \(4.3\pm0.47\) mmHg after bethanechol to \(2.2\pm0.20\) mmHg after the \(4^{th}\) dose (0.016 mg/kg) and remained decreased until 60 min after the last dose (2.7±0.33 mmHg). In the other groups, the decrease of Paw after bethanechol was not statistically significant \((p>0.006)\) at any dose or time period, although there was a tendency to decrease especially in the atropine group \((p=0.04)\). No treatment group at any dose or period differed significantly from the vehicle group \((p>0.006)\).

### 3.3.2.4 Bronchial Pressure Changes:

Bronchial pressure was less affected than other parameters in all of the treatment groups at any dose or time period, although there was a slight decrease, especially, in terbutaline group (Figure 3.12 and Tables 3.1, 3.2, and 3.3). No significant \((p>0.006)\) differences were found between treatments and the vehicle groups due to the high variance among the animals.

### 3.3.2.5 Pulmonary Compliance Changes:

The bethanechol-induced bronchoconstriction decreased pulmonary compliance (PC) or increased the lung and airways stiffness significantly \((p=0.002)\) in terbutaline group only \((51.8\pm12.35\) ml/mmHg) compared to the baseline \((98.1\pm12.22\) ml/mmHg) (Figure 3.13 and Table 3.3). The decrease in PC was not significant in the vehicle, aminophylline, or atropine groups \((p\) values are: 0.06, 0.14, and 0.03, respectively) (Tables 3.1 and 3.2). Terbutaline increased PC or relieved stiffness significantly at \(3^{rd}\) dose \((91.3\pm6.99\) ml/mmHg, \(p=0.006)\) and \(4^{th}\) dose \((93.3\pm14.10\) ml/mmHg, \(p=0.004)\) compared to the decreased PC after bethanechol. At 10, 30, and 60 min after the last dose of terbutaline, PC decreased to the values that did not differ from either the baseline or
after bethanechol (p>0.006). No group of any bronchodilator was different from the vehicle group (p>0.006). The effect of bethanechol on PC was very brief and this parameter was very variable, high SE, among the animals in each group at the baseline.

### 3.3.2.6 Blood gases, pH, and End-Tidal gases Changes:

Bethanechol increased blood PCO₂ insignificantly (p>0.006) from the baseline in all of the groups (Figure 3.14). The range of PCO₂ at the baseline was 33.5±1.44 - 37.5±1.19 mmHg and increased after bethanechol to 38.5±6.51 - 46.0±3.39 mmHg (Tables 1.3, 2.3, and 3.3). No dose or period of time was different from the baseline or after bethanechol values in all of the groups except in the vehicle group where there were significant (p<0.006) decreases in PCO₂ at 4th dose, 10, 30, and 60 min after the last dose compared to bethanechol.

Blood PO₂ decreased significantly (p<0.006) after bethanechol (44.5±7.71-57.3±6.42 mmHg) compared to the baseline (82.5±6.96 - 99.8±6.47 mmHg) in all groups (Figure 3.15 and Tables 1.3, 2.3, and 3.3). It increased after the first dose of the three bronchodilators to near the baseline values; however, in the vehicle group at the first and second doses PO₂ was significantly (p<0.006) different from the baseline. No differences were found among the four groups at any dose or time period.

Blood pH decreased to 7.2±0.04 after bethanechol; however, this reduction was significant (p<0.006) in the vehicle (after bethanechol and first dose), atropine, and terbutaline groups (after bethanechol) and not significant in the aminophylline group compared to the baseline (Figure 3.16 and Tables 1.3, 2.3, and 3.3). Starting at the first dose until the end of the experiment, all the bronchodilators increased pH to the values that did not differ from the baseline.
End-tidal PCO$_2$ did not change after bethanechol or at any dose/time after bronchodilators, although there was a nonsignificant (p>0.006) increase in atropine group. Compared to the vehicle group, the atropine group only at 60 min after the last dose differed significantly (p=0.002) (Figure 3.17 and Table 1.3, 2.3, and 3.3). End-tidal oxygen percentage (O$_2$%) did not change at any dose/time and no differences were observed between the treatments and the vehicle groups (p>0.006).

In summary, the significant bronchodilatory and hemodynamic effects of atropine after bethanechol-induced bronchoconstriction were greater than to those of aminophylline and terbutaline. Atropine relieved the bronchospasm caused by bethanechol and decreased Tp with the greatest effect compared to the other treatments. Although atropine accelerated HR, it reduced Pap, LVEDP, and did not change either CO or PVR. Like the other bronchodilators, atropine corrected the abnormal changes in blood gases and pH that were caused by bethanechol.

The bronchodilatory effects of terbutaline were more pronounced in decreasing Paw and increasing PC after the bronchoconstriction. Although terbutaline did not alter HR, it increased Pap after the highest dose without any changes in LVEDP, CO, or PVR.

Aminophylline was the least effective bronchodilator on both respiratory and hemodynamic parameters, although it decreased LVEDP and tended to increase HR and PVR after the highest dose.
3.4 DISCUSSION AND CONCLUSIONS:

The purpose of this study was to evaluate the respiratory and hemodynamic effects of three well known bronchodilators: aminophylline, atropine, and terbutaline. The induction of bronchoconstriction by bethanechol was chosen because of the significant tracheo-bronchial and hemodynamic changes due to stimulating parasympathetic receptors in the cardio-respiratory system (see Chapter 2).

These experiments demonstrated that atropine, a nonselective muscarinic receptor antagonist, is the most effective bronchodilator in reversing the tracheo-bronchial spasm induced by bethanechol. Atropine also relieved the vasoconstriction of pulmonary artery and decreased left ventricular end-diastolic pressure without changing cardiac output or pulmonary vascular resistance. These respiratory and hemodynamic modifications as well as the improvement in blood gases promote the correction of ventilation/perfusion ratio that was diminished by bronchoconstriction. The other two bronchodilators did not have prolonged effects on the tracheo-bronchial system, possibly because of the unusual intravenous administration, especially of terbutaline which is usually delivered by inhalation (Svedmyr and Simonsson, 1981). Terbutaline, a selective $\beta_2$ agonist, increased the total lung compliance by decreasing airways pressure which improves ventilation of the lung and airways due to the abundance of $\beta_2$-adrenoceptors in small airways (see below). Aminophylline, a phosphodiesterase inhibitor, had a few and transient effects on both respiratory and hemodynamic parameters, presumably, because of the bolus injection compared to infusion (Svedmyr and Simonsson, 1981).

Studies of the airways innervation are very extensive and have been carried out to explain different abnormalities and phenomena such as asthma and other
bronchoconstrictive diseases in order to determine the appropriate and effective bronchodilators (Cabezas et al., 1971; Leff et al., 1983; Woolcock et al., 1969; and Stone et al., 1973). Barnes et al., 1983, conducted one of the innovative works that determined the autonomic receptors in airway smooth muscle of ferret by using autoradiographic localization method. They found that cholinergic receptors were numerous in large bronchial smooth muscle and almost absent in the distal bronchioles; whereas, β and α adrenoceptors were in high density in small bronchioles and they are rare in large airways. The striking differences of variability in receptor distribution among different airway segments might explain the inconsistent responses to several bronchodilators along the respiratory tract. The majority of researchers agree that parasympathetic tone is the predominant controller of tracheo-bronchial smooth muscle and that sympathetic innervation balances this excitatory influence in dogs. However, sympathetic stimulation does not completely inhibit vagal constriction and the dilatory effect of this stimulation only occurred after asphyxia (Cabezas et al., 1971). The important outcome of the previous study is that sympathetic involvement in bronchodilation depends on intact vagal innervation because sympathetic stimulation or injection of isoproterenol did not cause significant bronchodilation in vagotomized dogs. Different changes in airways resistance in responses to vagal stimulation and β-adrenergic blockade demonstrated that propranolol caused increase in peripheral (small) greater than central (large) airways resistance; whereas, vagal stimulation produced same effect in the opposite sites (Woolcock et al., 1969). In addition, vagal stimulation, propranolol, or a combination did not change the total dynamic compliance of the lung, which is compatible with the results of the present study. In another study, Weinmann and coworkers, 1985, found that β-
sympathetic receptors in the lung periphery are under tonic stimulation in anesthetized dogs from circulating adrenal catecholamines. The lack of atropine effect on increased resistance through collateral system (Rcs) by propranolol indicates the dominance of $\beta_2$-adrenceptors over muscarinic receptors in the peripheral airways (Weinmann et al., 1985). In the present results, bethanechol failed to change bronchiolar pressure and insignificantly decreased pulmonary compliance (Figures 3.12 and 3.13); hence atropine did not have appreciable effects on these parameters. *In situ* comparison of cholinergic and adrenergic contractile responses in the canine trachea and bronchus revealed that there is a substantial heterogeneity in the physiological and pharmacological cholinergic and $\alpha$-adrenergic tracheo-bronchoconstriction properties with the latter being involved in stronger constriction of the bronchi more than of the trachea (Leff et al., 1983). Of the sympathetic control of airways smooth muscle, there has been an agreement that $\beta$-adrenoceptors have more profound role in bronchodilation than the constrictive influence of $\alpha$-adrenoceptors in peripheral airways. Stimulation of $\beta$-adrenoceptors by terbutaline relaxed distal more than proximal intraparenchymal airways in dogs, which is likely ascribed to the differences in receptor density in various segments of the airway (Akhtar et al., 1999). Although it is beyond the scope of the present study, it should be noted that the existence of $\alpha$-adrenoceptors in normal airways is very controversial. Some investigators found no evidence of $\alpha$-adrenoceptors effect on airway diameter after blockade of $\beta$-receptors and sympathetic stimulation (Cabezas et al., 1971). In human, norepinephrine, an $\alpha$-adrenergic agonist, did not affect airways conductance, indicating absence or inactive $\alpha$-adrenoceptors in the lower airways (Stone et al., 1973). Nevertheless, in asthma where there is an excessive release of inflammatory mediators,
α-adrenoceptors could initiate a contractile response by post-receptor mechanism and also vasoconstrict pulmonary arterioles to correct ventilation/perfusion ratio (Barnes et al., 1983 and 1995).

The role of parasympatholytics in bronchoconstriction has been reviewed by Morley, 1994, and he stated that M₃ muscarinic receptors mediate bronchospasm in asthma. The bronchodilatory effect of atropine in the present experiments is consistent with the physiological studies in which a greater bronchoconstriction was elucidated in larger rather than smaller airways after vagal stimulation or parasympathomimetics in vivo (Colebatch et al., 1966) and in vitro (Drazen and Schneider, 1978). The present results lead to the same conclusion reached by Barnes, 1995, in that cholinergic mechanism may play a major role in asthma exacerbations because atropine rapidly reversed the bethanechol-induced bronchospasm. In addition, these results confirmed the observation of parasympatholytic suppression of airways obstruction following methacholine inhalation in asthmatics and this effect was due to M₃ muscarinic receptors antagonism (Gross and Skorodin, 1984), which also abrogates mucosal secretion (Morley, 1994). Therefore, it is believed that there is a strong suggestion of therapeutic potential of parasympatholytics in the remedy of, at least, acute type of asthma. It should be noted, however, that nonselective antimuscarinic bronchodilators may trigger other side effects such as tachycardia as shown above with atropine. The nonselective antagonism of autoregulatory presynaptic (neural) M₂ muscarinic receptors, which regulate acetylcholine release, by low doses of ipratropium decreases airway size due to increased acetylcholine release in postsynaptic junction, which in turn activates post-junctional M₃ muscarinic receptors (Groeben and Brown, 1996). This paradoxical
bronchoconstriction of ipratropium accounts for exacerbations of asthma and chronic obstructive pulmonary disease (COPD) symptoms that have been reported in some patients (O’Callaghan et al., 1989; and Mann et al., 1984). Nonetheless, in relatively large doses, ipratropium blocks M₃ receptors on airways smooth muscle which promotes bronchodilation and decreases mucous secretion.

The previous investigations thoroughly demonstrated the effects of different bronchodilators on respiratory system; however, few were concerned about the hemodynamic effects other than heart rate or blood pressure. It is important to assess the cardiac accelerating and vasodepressing effects of different bronchoactive drugs (Bowman and Raper, 1976). Also, it is crucial to monitor other hemodynamic changes since some bronchodilators possess cardiac stimulatory effect such as the positive inotropic effect of aminophylline (Matthay, 1987). The lack of specificity of atropine, especially in high doses, increased heart rate due to the blockade of postsynaptic cardiac M₂ muscarinic receptors of sinoatrial (S.A) node (Micheletti et al., 1987). The other hemodynamic effects of atropine including the reduction in Pap and LVEDP without changing cardiac output or pulmonary vascular resistance are reported for the first time in the present study. Nonselective β₂ and β₁ agonists, such as isoproterenol, dramatically increased heart rate and cardiac output with large decrease in pulmonary vascular resistance and a small decrease in pulmonary arterial pressure in a methacholine-challenged canine bronchoconstriction model (Rodriguez-Roisin et al., 1984). In the same model, salbutamol, a selective β₂ agonist, caused nonsignificant changes and was less effective on the above parameters, except a small increase in pulmonary artery pressure. These findings are in accordance with the terbutaline effects in the present
Intravenous infusion of aminophylline or oral administration of theophylline in humans increase heart rate, right and left ventricular ejection fractions, and cardiac index (variably), while decrease pulmonary artery pressure, pulmonary vascular resistance, and right and left ventricular end-diastolic pressures (Matthay, 1987). In the present study, after the cumulative dose of 20 mg/kg aminophylline, heart rate increased compared to the vehicle group with a tendency to decrease the pulmonary arterial pressure. In addition, there was a late, but significant, decrease in left ventricular end-diastolic pressure and slight increases or no changes in both cardiac output and pulmonary vascular resistance. Positive inotropic and chronotropic actions of aminophylline are due to direct alteration of intracellular Ca\(^{2+}\) concentration by increasing calcium influx and to indirect inhibition of phosphodiesterase (PDE), which increases cAMP.

Extensive evaluations of therapeutic benefits and side effects of aminophylline and β\(_2\) agonists alone or in combination have been reported previously (Wolfe et al., 1978; Akhtar et al., 1999; Bowman and Raper, 1976; and Stepanek, 1978). The short duration and decreased effectiveness of these bronchodilators in the present study are in conflict with the findings of others. However, some of these effects, especially hemodynamics, are consistent with the typical activation of adrenoceptors and the inhibition of phosphodiesterase. Filbert et al., 1992, found that norepinephrine, salbutamol, and isoproterenol caused a transient and not sustained relaxation of canine tracheal smooth muscle pre-contracted by soman, an acetylcholinesterase inhibitor, which is similar to what has been shown after terbutaline in the present work. On the other hand, low concentrations of antimuscarinics agents such as atropine and scopolamine prevented and reversed the increased tension of tracheal smooth muscle, as is evident in the present work.
results, indicating parasympathetic dominance of large airways control. In vivo model of acetylcholine-induced (by inhalation) bronchospasm was used to challenge the bronchodilatory effect of terbutaline which inhibited 10-70% of bronchoconstriction after inhalation of 0.05-0.5 mg/kg (Stepanek, 1978). These high doses of terbutaline only lasted for 2 hr and caused large increase in heart rate; whereas, 0.04 mg/kg ipratropium bromide inhalation inhibited 95% of the bronchospasm with only transient changes in heart rate. Intravenous injection of 0.04 mg/kg of atropine was very effective in reversing bethanechol-induced tracheo-bronchial constriction in the present study, although heart rate was elevated.

The difference in bronchodilatory effects between \( \beta_2 \)-agonists and antimuscarinics could be explained by binding of two different regulatory G proteins to adrenergic and muscarinic receptors (Torphy et al., 1985). While \( \beta_2 \)-adrenoceptors stimulation causes binding to stimulatory G protein (G\( s \)), which activates adenylyl cyclase (AC) and consequently increases cAMP, muscarinic receptors coupled to a regulatory G protein – subtype q/11- (G\( q/11 \)), which stimulates phospholipase C (PLC) that hydrolyzes phosphatidylinositol 4,5 biphosphate (PIP\(_2\)) (Figure 1.4). Increased cAMP, a second messenger, activates protein kinase A (PKA) which in turn phosphorylates myosin light chain kinase (MLCK) to inactive form that promotes relaxation (Hoffman, 1998). On the other hand, hydrolysis of PIP\(_2\) results in two second messengers: the first is inositol 1,4,5 triphosphate (IP\(_3\)) which releases Ca\(^{+2}\) from sarcoplasmic reticulum and the second is diacylglycerol (DAG) which activates protein kinase C (PKC) that opens Ca\(^{+2}\) channels of smooth muscle (Pappano, 1998). This functional antagonism of sympathetic-induced relaxation and parasympathetic-induced contraction and the ability of muscarinic
receptors to suppress biochemical events of adrenergic relaxation could account for the reduced efficacy of $\beta_2$ agonists’ bronchodilation. Alternatively, the density of muscarinic receptors is higher than those of $\beta_2$ receptors (see above) and activation of a greater number of adrenergic receptors is required to overcome the bronchoconstrictive action of muscarinic receptors. Another explanation of this decreased efficacy of adrenergic bronchodilation is the desensitization of $\beta_2$ receptors to agonists, a well known phenomenon which is thought to be due to uncoupling between $\beta_2$ receptors and AC (Harden, 1988; and Tobias et al., 1990). Desensitization has been recently proposed and is defined as a rapid decrease in response upon repeated receptor stimulation or antagonism; this observation merits further investigation.

The discrepancy between the present findings and those of others in respect to the respiratory effects of terbutaline and aminophylline is a consequence of different experimental settings and protocols. First, these two different bronchodilators are administered orally or by inhalation for terbutaline and orally sustained release or continuous infusion for aminophylline (Karpel et al., 1994). The purpose of intravenous bolus injection of the three bronchodilators investigated here was to eliminate factors that alter absorption from the mucosa of gastrointestinal and respiratory systems, which results in low plasma concentrations (Cp). Furthermore, delivering drugs directly to blood stream is the best way to assess hemodynamic changes because of equal distribution of these agents. Second, the effects of these bronchodilators were monitored for 60 minutes after the last dose, while mean half-life ($t_{1/2}$) of theophylline is 5.7 hours and that of terbutaline is 2-6 hours (Mckiernan et al., 1981; and Paul, 2002). Third, the dosage regimens followed in the present protocol was to determine the optimal dose that
gives desirable bronchodilatory effect with minimal side effects; this is different from those of other studies and from the clinical practice. Fourth, besides variations in responses to the same bronchodilator between species, anesthetic choice may affect both respiratory and hemodynamic parameters at the baseline, as the case with pentobarbital versus chloralose (see Chapter 2). The above limitations of the present findings necessitate extra caution when these results are extrapolated to different conscious species or to patients with pre-existing congenital cardiopulmonary diseases.

These findings suggest that neither $\beta_2$ agonism nor inhibition of PDE were able to revere bethanechol-induced tracheo-bronchial constriction and muscarinic antagonism was very effective in relaxing airway smooth muscle with some favorable hemodynamic effects. Accordingly, cholinergic control of the respiratory system is tonic and predominant, at least, in larger airways and there is a potential therapeutic benefit of parasympatholytics in bronchospasm that should be reconsidered. In addition, this model of bronchospasm might be used in evaluating mechanisms of other bronchodilators that alter autonomic control of airways smooth muscle as well as hemodynamics. Particular interest should be directed to the effects of new and selective M$_3$ antimuscarinics on smooth muscle reactivity and mucus secretion in COPD and asthma.
Figure 3.1: The Hemodynamic and Respiratory Effects of the Fourth Intravenous Cumulative Dose of Aminophylline (20 mg/kg) after Bethanechol-induced Bronchoconstriction:
Note the slight and transient decrease in Tp and moderate increase in LVP after the injection.
Table 3.1: Changes in Respiratory and Hemodynamic Parameters in Response to the Bronchodilatory Effects of Cumulative Doses (2.5, 5, 10, and 20 mg/kg) of Aminophylline after Bethanechol-Induced Bronchoconstriction:
Mean ± SEM of 4 dogs. For statistical significance values see graphs.

<table>
<thead>
<tr>
<th>Dose/Time</th>
<th>Heart Rate (bpm)</th>
<th>( T_p ) (mmHg)</th>
<th>Paw (mmHg)</th>
<th>Ecp (mmHg)</th>
<th>Pcp (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>Cardiac Output (l/min)</th>
<th>Pul. Vascular Resistance ( LVEDP-Pcp/CO )</th>
<th>Pul. Compliance ( Vc/Paw )</th>
<th>Blood Gases</th>
<th>End Tidal CO(_2)</th>
<th>O(_2)%</th>
<th>Peak effect on ( T_p ) (mmHg)</th>
<th>Time to Peak effect on ( T_p ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>96.5 ± 12.66</td>
<td>19.5 ± 0.45</td>
<td>1.7 ± 0.52</td>
<td>29.9 ± 0.98</td>
<td>15.0 ± 0.66</td>
<td>13.7 ± 1.14</td>
<td>1.6 ± 0.17</td>
<td>1.3 ± 0.57</td>
<td>153.1 ± 8.87</td>
<td>37.5 ± 1.19</td>
<td>38.8 ± 2.14</td>
<td>7.4 ± 0.02</td>
<td>39.5 ± 0.96</td>
<td>19.5 ± 0.29</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>83.4 ± 28.56</td>
<td>51.8 ± 5.77</td>
<td>3.0 ± 0.61</td>
<td>29.9 ± 1.36</td>
<td>21.8 ± 1.34</td>
<td>16.3 ± 2.56</td>
<td>1.6 ± 0.50</td>
<td>3.1 ± 0.73</td>
<td>80.4 ± 25.92</td>
<td>38.5 ± 6.51</td>
<td>53.5 ± 3.30</td>
<td>7.2 ± 0.05</td>
<td>39.3 ± 2.29</td>
<td>19.3 ± 0.25</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>121.5 ± 10.63</td>
<td>58.4 ± 2.83</td>
<td>2.0 ± 0.44</td>
<td>29.6 ± 1.54</td>
<td>20.3 ± 1.57</td>
<td>12.4 ± 1.83</td>
<td>1.9 ± 0.24</td>
<td>4.1 ± 0.31</td>
<td>113.1 ± 24.45</td>
<td>36.3 ± 4.31</td>
<td>71.5 ± 5.68</td>
<td>7.3 ± 0.06</td>
<td>40.0 ± 2.80</td>
<td>19.3 ± 4.74</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>131.4 ± 10.10</td>
<td>59.5 ± 1.85</td>
<td>1.8 ± 0.46</td>
<td>29.6 ± 1.77</td>
<td>19.3 ± 2.10</td>
<td>11.2 ± 0.47</td>
<td>1.8 ± 0.20</td>
<td>4.4 ± 0.92</td>
<td>136.4 ± 34.98</td>
<td>31.3 ± 6.17</td>
<td>78.5 ± 5.87</td>
<td>7.3 ± 0.05</td>
<td>38.8 ± 3.64</td>
<td>19.3 ± 1.64</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>137.0 ± 12.78</td>
<td>57.1 ± 1.79</td>
<td>1.7 ± 0.48</td>
<td>29.1 ± 1.74</td>
<td>18.3 ± 1.83</td>
<td>11.4 ± 0.53</td>
<td>1.8 ± 0.23</td>
<td>3.9 ± 0.98</td>
<td>143.0 ± 31.02</td>
<td>31.5 ± 5.63</td>
<td>80.3 ± 6.30</td>
<td>7.4 ± 0.05</td>
<td>38.0 ± 3.72</td>
<td>19.3 ± 1.99</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>152.9 ± 11.36</td>
<td>54.1 ± 2.26</td>
<td>1.6 ± 0.53</td>
<td>29.1 ± 1.90</td>
<td>18.0 ± 1.62</td>
<td>10.9 ± 0.22</td>
<td>1.9 ± 0.14</td>
<td>3.9 ± 0.20</td>
<td>162.0 ± 40.55</td>
<td>34.5 ± 3.66</td>
<td>75.8 ± 6.09</td>
<td>7.4 ± 0.05</td>
<td>38.5 ± 3.71</td>
<td>19.0 ± 4.37</td>
</tr>
<tr>
<td>10 min</td>
<td>146.6 ± 12.87</td>
<td>52.1 ± 2.96</td>
<td>1.6 ± 0.56</td>
<td>28.8 ± 1.95</td>
<td>17.5 ± 1.67</td>
<td>10.3 ± 0.34</td>
<td>1.7 ± 0.26</td>
<td>4.4 ± 1.25</td>
<td>170.4 ± 45.04</td>
<td>34.5 ± 4.17</td>
<td>77.0 ± 7.11</td>
<td>7.4 ± 0.05</td>
<td>38.8 ± 3.47</td>
<td>19.0 ± 4.00</td>
</tr>
<tr>
<td>30 min</td>
<td>133.7 ± 19.13</td>
<td>49.7 ± 2.97</td>
<td>1.8 ± 0.44</td>
<td>28.6 ± 2.16</td>
<td>18.0 ± 1.73</td>
<td>10.9 ± 0.96</td>
<td>1.6 ± 0.28</td>
<td>4.7 ± 1.69</td>
<td>130.3 ± 27.47</td>
<td>34.8 ± 4.03</td>
<td>76.0 ± 5.57</td>
<td>7.4 ± 0.05</td>
<td>39.0 ± 3.81</td>
<td>19.0 ± 4.00</td>
</tr>
<tr>
<td>60 min</td>
<td>130.5 ± 18.80</td>
<td>47.3 ± 2.93</td>
<td>1.7 ± 0.49</td>
<td>27.9 ± 2.57</td>
<td>18.5 ± 1.38</td>
<td>10.9 ± 1.42</td>
<td>1.6 ± 0.36</td>
<td>5.7 ± 2.15</td>
<td>140.4 ± 30.65</td>
<td>33.0 ± 5.43</td>
<td>80.5 ± 9.47</td>
<td>7.4 ± 0.04</td>
<td>39.8 ± 4.27</td>
<td>19.5 ± 4.29</td>
</tr>
</tbody>
</table>
Figure 3.2: The Hemodynamic and Respiratory Effects of the Second Intravenous Cumulative Dose of Atropine (0.04 mg/kg) after Bethanechol-induced Bronchoconstriction:
Note the drastic and persisted decrease in Tp and moderate decrease in Pap. HR increased significantly after the injection.
<table>
<thead>
<tr>
<th>Dose/Time</th>
<th>Heart Rate (bpm)</th>
<th>Tp (mmHg)</th>
<th>Paw (mmHg)</th>
<th>Bcp (mmHg)</th>
<th>Pap (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>Cardiac Output (l/min)</th>
<th>Pul. Vascular Resistance (LVEDp-Pap/CO)</th>
<th>Pul. Compliance (Vc/Paw)</th>
<th>Blood Gases</th>
<th>pH</th>
<th>End Tidal</th>
<th>Peack effect on Tp (mmHg)</th>
<th>Time to Peack effect on Tp (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>84.3 ± 13.06</td>
<td>19.2 ± 0.61</td>
<td>1.1 ± 0.39</td>
<td>29.1 ± 0.87</td>
<td>14.4 ± 1.28</td>
<td>13.9 ± 0.68</td>
<td>1.5 ± 0.19</td>
<td>1.3 ± 0.46</td>
<td>314.5 ± 121.72</td>
<td>36.3 ± 4.00</td>
<td>82.5 ± 6.56</td>
<td>7.4 ± 0.04</td>
<td>36.5 ± 0.29</td>
<td>19.0 ± 0.00</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>70.4 ± 16.78</td>
<td>50.1 ± 0.87</td>
<td>2.2 ± 0.31</td>
<td>28.8 ± 1.25</td>
<td>22.7 ± 1.59</td>
<td>18.9 ± 0.78</td>
<td>2.0 ± 0.82</td>
<td>3.5 ± 0.04</td>
<td>98.4 ± 23.10</td>
<td>42.3 ± 3.33</td>
<td>52.5 ± 5.95</td>
<td>7.2 ± 0.04</td>
<td>40.0 ± 1.41</td>
<td>19.3 ± 0.25</td>
</tr>
<tr>
<td>0.02 mg/kg</td>
<td>103.2 ± 3.82</td>
<td>49.1 ± 1.2</td>
<td>1.2 ± 0.39</td>
<td>28.4 ± 1.56</td>
<td>15.7 ± 1.25</td>
<td>14.0 ± 0.04</td>
<td>1.8 ± 0.45</td>
<td>218.3 ± 53.14</td>
<td>36.3 ± 0.03</td>
<td>72.5 ± 6.98</td>
<td>7.3 ± 0.05</td>
<td>39.3 ± 2.98</td>
<td>19.0 ± 0.04</td>
<td>44.25 ± 1.36</td>
</tr>
<tr>
<td>0.04 mg/kg</td>
<td>165.4 ± 5.53</td>
<td>27.1 ± 1.2</td>
<td>1.2 ± 0.35</td>
<td>28.0 ± 1.72</td>
<td>16.1 ± 1.17</td>
<td>12.3 ± 0.93</td>
<td>2.0 ± 0.45</td>
<td>223.2 ± 67.26</td>
<td>37.8 ± 2.72</td>
<td>76.0 ± 4.67</td>
<td>7.3 ± 0.04</td>
<td>41.0 ± 2.42</td>
<td>19.0 ± 0.14</td>
<td>25.42 ± 2.18</td>
</tr>
<tr>
<td>0.08 mg/kg</td>
<td>178.5 ± 3.21</td>
<td>18.4 ± 1.68</td>
<td>1.2 ± 0.35</td>
<td>27.7 ± 1.87</td>
<td>16.7 ± 1.23</td>
<td>11.8 ± 1.03</td>
<td>2.0 ± 0.42</td>
<td>226.5 ± 69.54</td>
<td>38.0 ± 6.94</td>
<td>77.3 ± 3.04</td>
<td>7.3 ± 0.04</td>
<td>41.8 ± 2.78</td>
<td>19.0 ± 0.16</td>
<td>18.13 ± 1.24</td>
</tr>
<tr>
<td>0.16 mg/kg</td>
<td>192.4 ± 4.79</td>
<td>18.2 ± 1.70</td>
<td>1.2 ± 0.34</td>
<td>27.3 ± 1.96</td>
<td>17.7 ± 1.22</td>
<td>11.8 ± 1.25</td>
<td>2.0 ± 0.44</td>
<td>210.6 ± 57.13</td>
<td>36.8 ± 14.11</td>
<td>79.5 ± 3.66</td>
<td>7.3 ± 0.04</td>
<td>43.3 ± 2.66</td>
<td>19.0 ± 0.18</td>
<td>17.45 ± 0.85</td>
</tr>
<tr>
<td>10 min</td>
<td>170.7 ± 5.67</td>
<td>18.0 ± 1.78</td>
<td>1.3 ± 0.33</td>
<td>27.2 ± 2.04</td>
<td>17.8 ± 1.16</td>
<td>12.0 ± 0.70</td>
<td>1.9 ± 0.39</td>
<td>189.4 ± 45.71</td>
<td>39.0 ± 13.49</td>
<td>78.8 ± 4.31</td>
<td>7.3 ± 0.04</td>
<td>43.3 ± 2.61</td>
<td>19.0 ± 0.15</td>
<td>17.45 ± 0.85</td>
</tr>
<tr>
<td>30 min</td>
<td>151.0 ± 5.46</td>
<td>17.7 ± 1.76</td>
<td>1.3 ± 0.37</td>
<td>27.0 ± 2.10</td>
<td>18.5 ± 1.28</td>
<td>12.6 ± 0.60</td>
<td>1.8 ± 0.41</td>
<td>199.2 ± 60.18</td>
<td>39.5 ± 13.10</td>
<td>79.3 ± 3.06</td>
<td>7.3 ± 0.04</td>
<td>42.8 ± 2.84</td>
<td>19.0 ± 0.19</td>
<td>17.45 ± 0.85</td>
</tr>
<tr>
<td>60 min</td>
<td>124.8 ± 5.30</td>
<td>17.2 ± 1.80</td>
<td>1.4 ± 0.33</td>
<td>26.6 ± 2.04</td>
<td>19.6 ± 1.35</td>
<td>13.9 ± 0.64</td>
<td>1.8 ± 0.43</td>
<td>173.7 ± 40.98</td>
<td>39.0 ± 13.70</td>
<td>77.8 ± 5.71</td>
<td>7.3 ± 0.04</td>
<td>44.0 ± 2.70</td>
<td>19.0 ± 0.19</td>
<td>17.45 ± 0.85</td>
</tr>
</tbody>
</table>

Table 3.2: Changes in Respiratory and Hemodynamic Parameters in Response to the Bronchodilatory Effects of Cumulative Doses (0.02, 0.04, 0.08, and 0.16 mg/kg) of Atropine after Bethanechol-Induced Bronchoconstriction: Mean ± SEM of 4 dogs. For statistical significance values see graphs.
Figure 3.3: The Hemodynamic and Respiratory Effects of the Fourth Intravenous Cumulative Dose of Terbutaline (0.016mg/kg) after Bethanechol-induced Bronchoconstriction:
Note the prompt and relatively transient decease in Tp. HR and LVP increased slightly; while, Pap increased significantly.
Table 3.3: Changes in Respiratory and Hemodynamic Parameters in Response to the Bronchodilatory Effects of Cumulative Doses (0.002, 0.004, 0.008, and 0.016 mg/kg) of Terbutaline after Bethanechol-Induced Bronchoconstriction:
Mean ± SEM of 4 dogs. For statistical significance values see graphs.

| Dose/Time | Heart Rate (bpm) | Tp (mmHg) | Paw (mmHg) | Bcp (mmHg) | Pao2 (mmHg) | LVEDP (mmHg) | Cardiac Output (l/min) | Pul. Vascular Resistance LVEDP/Pao2 | Pul. Compliance Vc/Pao2 | Blood Gases | pH | End Tidal PCO2 | O2% | Peak effect on Tp (mmHg) | Peak effect on Tp (min) |
|-----------|------------------|-----------|------------|------------|-------------|---------------|------------------------|-----------------------------------|------------------------|-------------|----------------|------|------------------------|---------------------|
| Baseline  | 73.9 ± 5.9       | 18.6 ± 3.4| 2.1 ± 0.22 | 29.4 ± 0.78| 12.2 ± 1.12 | 15.8 ± 0.56    | 2.0 ± 0.34              | 2.1 ± 0.22                       | 98.1 ± 12.22                        | 33.5 ± 1.44 | 99.8 ± 6.47 | 7.4 ± 0.02 | 34.8 ± 0.48 | 19.5 ± 0.29 |
| 0.5 mg/kg | 102.0 ± 19.39    | 66.0 ± 2.93| 4.3 ± 0.47 | 28.6 ± 1.17| 22.0 ± 1.59 | 19.0 ± 0.47    | 2.1 ± 0.27              | 2.4 ± 0.27                       | 51.8 ± 12.35                        | 41.5 ± 0.66 | 57.3 ± 6.42 | 7.2 ± 0.05 | 37.5 ± 1.50 | 19.0 ± 0.00 |
| 0.002 mg/kg| 102.1 ± 5.69    | 66.4 ± 3.63| 2.7 ± 0.27 | 27.8 ± 1.50| 16.2 ± 1.29 | 15.6 ± 0.46    | 2.0 ± 0.27              | 0.7 ± 0.16                       | 78.0 ± 9.77                         | 34.0 ± 3.08 | 78.0 ± 5.32 | 7.3 ± 0.10 | 35.8 ± 1.70 | 19.0 ± 0.00 |
| 0.004 mg/kg| 94.8 ± 8.32     | 66.1 ± 5.63| 2.6 ± 0.45 | 27.0 ± 1.69| 16.1 ± 1.91 | 17.0 ± 0.47    | 2.0 ± 0.27              | 1.3 ± 0.16                       | 81.8 ± 6.77                         | 32.5 ± 4.13 | 85.8 ± 3.20 | 7.3 ± 0.03 | 35.5 ± 1.55 | 19.0 ± 0.00 |
| 0.008 mg/kg| 100.1 ± 9.01    | 63.4 ± 6.83| 2.3 ± 0.31 | 26.5 ± 1.85| 16.8 ± 1.92 | 17.5 ± 0.41    | 2.1 ± 0.27              | 1.2 ± 0.28                       | 91.3 ± 6.99                         | 33.0 ± 3.08 | 82.8 ± 7.79 | 7.3 ± 0.04 | 38.0 ± 1.83 | 19.0 ± 0.01 |
| 0.016 mg/kg| 112.6 ± 7.64    | 58.3 ± 7.92| 2.2 ± 0.20 | 26.0 ± 1.85| 18.8 ± 1.55 | 18.2 ± 0.38    | 2.4 ± 0.20              | 0.8 ± 0.14                       | 93.3 ± 14.10                        | 35.8 ± 5.57 | 77.8 ± 5.30 | 7.3 ± 0.03 | 41.3 ± 2.39 | 19.0 ± 0.00 |
| 10 min    | 94.4 ± 8.32     | 56.8 ± 9.45| 2.6 ± 0.41 | 25.3 ± 1.17| 20.1 ± 1.47 | 18.6 ± 0.27    | 2.0 ± 0.27              | 0.8 ± 0.27                       | 79.3 ± 8.44                         | 35.0 ± 2.48 | 81.3 ± 3.33 | 7.3 ± 0.03 | 40.3 ± 2.78 | 19.0 ± 0.00 |
| 30 min    | 86.1 ± 10.97    | 53.9 ± 12.31| 2.8 ± 0.43 | 24.2 ± 1.65| 19.6 ± 1.27 | 18.2 ± 0.50    | 1.7 ± 0.27              | 1.2 ± 0.31                       | 74.9 ± 5.47                         | 32.5 ± 3.48 | 84.3 ± 2.75 | 7.4 ± 0.04 | 38.0 ± 2.03 | 19.3 ± 0.25 |
| 60 min    | 83.3 ± 11.45    | 49.8 ± 12.98| 2.7 ± 0.33 | 23.4 ± 1.39| 20.4 ± 1.28 | 17.9 ± 0.28    | 1.6 ± 0.28              | 2.5 ± 0.62                       | 76.2 ± 4.67                         | 31.5 ± 3.30 | 84.3 ± 1.17 | 7.4 ± 0.04 | 36.3 ± 2.29 | 19.0 ± 0.00 |
Figure 3.4: The effects of Different Bronchodilators on Heart Rate after Bethanechol-Induced Bronchoconstriction:
No significant changes in HR after bethanechol compared to the baseline in all groups. Atropine, starting at 2\textsuperscript{nd} dose until the end of 60 min, increased HR significantly compared to the baseline. Aminophylline, starting at 4\textsuperscript{th} dose, and atropine differed significantly from the vehicle group. (*) and (‡) indicate p<0.006 compared to the baseline and vehicle group, respectively.
Figure 3.5: The effects of Different Bronchodilators on Pulmonary Arterial Pressure after Bethanechol-Induced Bronchoconstriction:
After bethanechol Pap increased significantly in all groups compared to the baseline; however, this increase lasted only in the vehicle group, at all doses, and terbutaline group, at the 4th dose. Atropine, at 1st, 2nd, and 3rd doses decreased Pap significantly compared to after bethanechol. Atropine and terbutaline differed significantly from the vehicle group at 1st dose. (*), ($$), and ($$) indicate p<0.006 compared to the baseline, after bethanechol, and vehicle group, respectively.
Figure 3.6: The effects of Different Bronchodilators on Left Ventricular End-diastolic Pressure after Bethanechol-Induced Bronchoconstriction:

After bethanechol LVEDP increased significantly only in the vehicle and atropine groups compared to the baseline. Compared to bethanechol, LVEDP decreased in the vehicle group, starting at 2nd dose, and in atropine group, starting at 1st dose, also in aminophylline group, at 10, 30, and 60 min after the last dose. No significant differences between the treatments and the vehicle. (*) and (§) indicate p<0.006 compared to the baseline, and after bethanechol, respectively.
Figure 3.7: The effects of Different Bronchodilators on Cardiac Output after Bethanechol-Induced Bronchoconstriction:
No significant changes in C.O occurred after bethanechol or at any time/dose period in all groups. No significant differences between the treatments and the vehicle.
Figure 3.8: The effects of Different Bronchodilators on Pulmonary Vascular Resistance after Bethanechol-Induced Bronchoconstriction:
No significant changes in P.V.R occurred after bethanechol or at any time/dose period in all groups. No significant differences between the treatments and the vehicle.
Figure 3.9: The effects of Different Bronchodilators on Tracheal Pressure after Bethanechol-Induced Bronchoconstriction:
Bethanechol increased Tp significantly in all groups compared to the baseline; however, Tp remained elevated in all groups except atropine group. Atropine, at 2\textsuperscript{nd} dose until the end of 60 min, decreased Tp significantly compared to after bethanechol. Only atropine group, starting at 2\textsuperscript{nd} dose, differed significantly from the vehicle group. (*) $(\$)$, and $(\ddagger)$ indicate $p<0.006$ compared to the baseline, after bethanechol, and vehicle group, respectively.
Figure 3.10: The Peak effects and Time to Peak of Different Bronchodilators on Tracheal Pressure after Bethanechol-Induced Bronchoconstriction:
The 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} doses of atropine were the most effective on Tp (bars) and they were, significantly, different from the 1\textsuperscript{st} dose; however, no statistical significant differences were found between the last three doses. Atropine group, starting at 2\textsuperscript{nd} dose, differed significantly from the other two bronchodilators and no significant difference between aminophylline and terbutaline groups at any dose. Within any group or among the three groups, no statistical significance differences in the times to peak (lines) were found. (*) and (‡) indicate $p<0.006$ compared to the first dose and to the other groups, respectively.
Figure 3.11: The effects of Different Bronchodilators on Airways Pressure after Bethanechol-Induced Bronchoconstriction:
Bethanechol increased Paw significantly only in the terbutaline group compared to the baseline; however, the nonsignificant increase in the other groups was because of high variability among the animals. Each dose of terbutaline decreased Paw significantly after bethanechol until the end of 60 min after the last dose. No differences between any treatment group and the vehicle. (*) and (§) indicate \( p<0.006 \) compared to the baseline and after bethanechol, respectively.
Figure 3.12: The effects of Different Bronchodilators on Bronchial Pressure after Bethanechol-Induced Bronchoconstriction:
No statistical significant differences were found after bethanechol, at any dose/time in any group, or among the groups.
Figure 3.13: The effects of Different Bronchodilators on Pulmonary Compliance after Bethanechol-Induced Bronchoconstriction:
Bethanechol decreased P.C significantly only in the terbutaline group compared to the baseline; however, in the other groups the decrease was nonsignificant because of high variability among the animals. The 3rd and 4th doses of terbutaline only increased P.C significantly compared to after bethanechol. No differences between any treatment group and the vehicle. (*) and (§) indicate p<0.006 compared to the baseline and after bethanechol, respectively.
Figure 3.14: The effects of Different Bronchodilators on Blood PCO₂ after Bethanechol-Induced Bronchoconstriction:
PCO₂ did not change after bethanechol in any group compared to the baseline; however, it decreased significantly in the vehicle group after the 4th dose until the end of 60 min compared to after bethanechol. No differences between any treatment group and the vehicle. (§) indicates p<0.006 compared to after bethanechol.
Figure 3.15: The effects of Different Bronchodilators on Blood $PO_2$ after Bethanechol-Induced Bronchoconstriction:

$PO_2$ decreased significantly after bethanechol in all groups compared to the baseline. The first dose of the three bronchodilators increased $PO_2$ to about the baseline value; therefore, no significant differences between baseline and after all doses of the treatments. In the vehicle group, $PO_2$ remained significantly low at 1$^{st}$ and 2$^{nd}$ doses compared to the baseline then increased to the normal values. No differences between any treatment group and the vehicle. (*) indicates $p<0.006$ compared to the baseline.
Figure 3.16: The effects of Different Bronchodilators on Blood pH after Bethanechol-Induced Bronchoconstriction:

Bethanechol decreased pH significantly in all groups, except aminophylline, compared to the baseline. After the first dose of the three bronchodilators, pH increased to about the baseline value; therefore, no significant differences between baseline and after all doses of the treatments. In the vehicle group, pH remained significantly lower than the baseline values at 1st dose then increased to the normal values. No differences between any treatment group and the vehicle. (*) indicates p<0.006 compared to the baseline.
Figure 3.17: The effects of Different Bronchodilators on End-Tidal PCO2 and O2% after Bethanechol-Induced Bronchoconstriction:
No significant changes in PCO2 (bars) or O2% (lines) after bethanechol or after any dose/time periods in any group. Only in atropine group, PCO2 at 60 min after the last dose differed significantly from the correspondent value in the vehicle group. (‡) indicates p<0.006 compared to the vehicle group.
3.5 LITERATURE CITED:


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CHAPTER 4

CONCLUSION AND PROSPECTIVE

4.1 GENERAL OUTCOMES:

This study has explored the effects of different tracheo-bronchoactive stimuli and their respiratory and hemodynamic contributions to the overall homeostasis in morphine/chloralose anesthetized dogs. Gas mixtures of hypercapnia and hypoxia had transient effects which were not sustained after termination of the stimulus and increasing the respiration frequency. Nonetheless, Hypoxia had more pronounced tracheal constriction and other hemodynamic effects than hypercarbia; however, this effect can be abolished by atropine (Iscoe and Fisher, 1995). Abnormal gas mixtures activate the central control of bronchomotion via central and peripheral chemoreceptors (afferent) which in turn activates the parasympathetic control of airways smooth muscle. Therefore, the third stimulus was aimed to a direct stimulation of the muscarinic receptors by bethanechol that caused provocative bronchoconstriction as well as profound hemodynamic effects, which may resemble the pathological conditions of asthma and chronic obstructive diseases COPD.

The other aspect of this study was comparing the respiratory and cardiovascular effects of three different classes of bronchodilators on bethanechol-induced tracheo-bronchoconstriction in dogs. Intravenous injection of 0.04 mg/kg atropine sulfate was
superior to the highest doses of 20 mg/kg aminophylline and 0.016 mg/kg terbutaline in reversing the bronchoconstriction. The failure of PDE inhibition and $\beta_2$-adrenoceptors agonism to reverse the bethanechol-induced tracheo-bronchial constriction could be due to the delivery methods of the drugs because they were given as an accumulation of 4 consecutive doses. Therefore, the same doses of aminophylline and terbutaline were given by continuous infusion to 2 dogs of each drug to test the effect of different drug delivery systems. These pilot studies revealed that intravenous infusion of aminophylline decreased Tp from 50 to 25 mmHg during 10 min of infusion and remained decreased until 40 min after infusion. On the other hand, terbutaline infusion decreased Tp from 65 to 45 mmHg only during infusion and was elevated back to prior infusion value after 10 min. Although, the effects of infusion were not analyzed quantitatively, both infused drugs increased HR, Pap, and LVP qualitatively. Therefore, drug delivery and duration of administration may influence the mechanism of action due to different pharmacokinetic properties of these drugs. Since each bronchodilator was monitored for 60 min after cumulative doses, different routes of drug administration for a longer period of time merits further investigation.

The broad conclusion one might draw from the above discussion is that parasympathetic control of smooth muscle reactivity is tonic and may not be obscured by adrenergic activation or PDE inhibition. Moreover, specific and highly selective M$_3$-muscarinic blockers, such as tiotropium and zamifenacin, could be of a particular importance in relaxing constricted airways in asthma and COPD (Hansel and Barnes, 2002; Disse, 2001; and Broadley and Kelly, 2001).
The other important finding of this research was determining the optimal bronchodilatory doses of the three drugs in dogs with minimal cardiovascular effects, since most veterinarians extrapolate, empirically, the dosing regimen from humans to animals.

4.2 UPDATES AND FUTURE DIRECTIONS:

As stated in the introduction of Chapter 3, the development of new techniques to explore different mechanism of diseases and therapeutic solutions is an ongoing endeavor. Modification of the sympathetic part of autonomic control in airways could be revealed after selective blockade of \( \alpha_1 \) and \( \beta_2 \)-adrenoceptors by prazosin and butoxamine, respectively, in the same experimental model above to evaluate their contribution to airways reactivity.

In the past, less attention was paid to the contribution of afferent (sensory) innervation in the pathogenicity of respiratory diseases due, in part, to inaccessibility of these nerves in intact animals and to the lack of specific blockers of their receptors. Nowadays, a particular interest has been directed to the afferent innervation role in modifying airways caliber through feedback mechanisms and many attempts of potential therapeutic approaches have been suggested. For example, direct inhibition of afferent nerves can be, experimentally, achieved by \( K^+ \) channel openers or activators such as cromakalim (Bowring et al., 1991) and dopamine which inhibits the ability of histamine to stimulate RAR (Jackson and Simpson, 2000).

Recently, there have been many speculations about the involvement of nonadrenergic noncholinergic (NANC) innervation in the pathological process of asthma because this system is thought to be the predominant inhibitory neural pathway in
humans (Canning and Fischer, 2001; and Barnes, 1984 and 1986). The neurotransmitters of NANC and sensory neuropeptides have recently been identified which include substance P, neurokinin A, VIP, and NO (Szarek and Spurlock, 1997; and Mitsufuji et al., 2001). Inhibitors of these tachykinins and blockers of neuropeptides receptors have been developed and their potential use as therapeutic agents is undergoing clinical trials in asthma. Examples of these are: NK₁-receptors selective antagonists, neuroendopeptidase, enkephalinase, opioid derivatives that inhibit the release of SP and depress the CNS, and gamma (γ) aminobutyric acid (GABA) that inhibits sensory transmission (Tables 1.1 and 1.2). Cromolyn and nedocromil sodium inhibit the release of bradykinin from activated sensory nerves and stabilize mast cells to prevent the release of inflammatory substances in asthma (Barnes 1995).

The current therapeutic strategies of asthma and COPD depend on the combination of many agents that bronchodilate airways and prevent the inflammation process (see Chapter 1). It is clinically recommended that patients should receive a combination of bronchodilators from different classes such as ipratropium, salbutamol and aminophylline in adjusted doses (Higgins, 2003; Friedman et al., 2002; Lu, 1997; Garrett, 1997; and Campbell, 2000). However, these combinations may exacerbate the cardiovascular effects, especially tachycardia, due to the synergistic mechanisms of bronchodilators. The new approaches to resolve this problem suggest the addition of a bradycardiac agent such as zatebradine which inhibits tachycardia induced by cAMP-dependent bronchodilators without affecting their bronchodilatory effects (Yamazaki et al., 1997).
Zatebradine inhibits hyperpolarization-activated inward currents (I_{f}) in the sinoatrial (SA) node which decreases the heart rate accordingly and is used to decrease tachycardia provoked by isoproterenol and aminophylline.

In addition, there is a trend in the pharmaceutical industry to develop more selective agents that only bind to particular receptors such as M₃ selective antagonists and could be combined with other drugs as a single dose in form of a “mini pill” or one puff. It follows, also, that some investigators were able to identify about 9 isoenzymes or subtypes of phosphodiesterase (PDE) in different organs with the preponderance of PDE3 and PDE4 in airways smooth muscle and inflammatory cells (Schudt et al., 1999). Thus, experimental agents that block these isoenzymes could be candidates for future therapeutic substitutes for aminophylline and theophylline (Hirota et al., 2001). Examples of these selective PDE inhibitors are motapizone and milrinone which inhibit PDE3, while rolipram and piclamilast inhibit PDE4. Moreover, both isoenzymes can be suppressed simultaneously by tolatfentrine or zardaverine which are mixed inhibitors of PDE3/4. Nevertheless, fatal arrhythmias could emerge with mono-selective PDE inhibitors, such as PDE3 inhibitor, resulting from an excessive cAMP in electrically unstable myocardium as manifested in ischemia/perfusion events in congestive heart failure (Schudt et al., 1999; and Heaslip et al., 1991). In heart failure, the down-regulation of pulmonary β-receptors and concomitant decrease in adenylyl cyclase (AC) activity result in a significant attenuation of cAMP-mediated airway relaxation (Borst et al., 1999). Therefore, it is necessary to evaluate the cardiovascular effects of dual-selective PDE3/4 inhibitors before considering their application in asthmatics with preexisting and congenital heart diseases.
Furthermore, clenbuterol, a more selective $\beta_2$-adrenceptors agonist, has been introduced with a unique inhibitory effect of cardiac $\beta_1$-adrenceptors; however, it is not approved for human use in the U.S because of the potential abuse as an anabolic drug (Prather et al., 1995 and Kuiper et al., 1998). It was approved by the FDA in 1998 to treat COPD in horses (Törneke et al., 1998; and Harkins et al., 2000) and its prescription is limited only to licensed veterinarians; however, chronic administration may negatively alter the cardiac functions (Sleeper et al., 2002). Agonists of $\beta$ adrenoceptors cause a fall in serum $K^+$ concentration with an increase in heart rate and prolong the QTc interval which may lead to the sudden death by provoking torsade de pointes (Wong et al., 1990). In addition, the chronic administration of long acting $\beta_2$ agonists, such as salbutamol and formoterol, may decrease the efficacy and response to short acting agonists because of receptors desensitization. Therefore, other bronchodilators should be considered in a combination regimen (Woude et al., 2001).

In conclusion, the best results of bronchodilation are achieved by a combination of selective agents that antagonize $M_3$-muscarinic receptors, inhibit PDE3/4, and activate $\beta_2$-adrenoceptors (Grootendorst et al., 2003). Accordingly, all these emerging drugs and new ideas need to be tested in animal models with induced bronchoconstriction by different stimuli before a large scale clinical testing. In the future, it is worthy to study the respiratory and hemodynamic effects of all these new therapeutic approaches such as afferent nerves interventions as well as these combinations and selective agonists using a standardized animal model.
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