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ALPHA$_1$ AND BETA ADRENERGIC RECEPTORS IN PONIES WITH RECURRENT OBSTRUCTIVE PULMONARY DISEASE

By

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ABSTRACT

ALPHA$_1$ AND BETA ADRENERGIC RECEPTORS
IN PONIES WITH RECURRENT
OBSTRUCTIVE PULMONARY DISEASE

By

Jacqueline Sue Scott

The pony suffers from a spontaneously occurring pulmonary disease (heaves) characterized by recurrent periods of airway obstruction accompanied by bronchial hyperresponsiveness similar to asthma. Alterations in alpha and beta adrenergic receptor function has been proposed as a factor in obstructive airway diseases such as asthma. To determine if abnormalities in the alpha$_1$ and beta adrenergic systems are involved in the airway obstruction seen with heaves, affected ponies (principals) and normals were studied after administration of adrenergic drugs. Pulmonary function measurements were made with principals in clinical remission (period A) and during airway obstruction (period B).

Aerosol phenylephrine (alpha$_1$ agonist) decreased dynamic compliance (Cdyn) and increased pulmonary resistance (RL) but prazosin (alpha$_1$ antagonist) did not cause bronchodilation in the principals. These data suggest that ponies with heaves when compared to controls have increased alpha$_1$ receptor responsiveness. However, this alteration in the alpha$_1$ system has a minimal role in the mechanism of heaves.

Ponies were treated with iv propranolol ($beta_{1\&2}$ antagonist) before receiving aerosol histamine. Beta blockade did not change histamine airway responsiveness, Cdyn, or RL in the principals at period A, but at period B iv propranolol increased RL only. To determine the effects of aerosol administration, principals were given aerosol propranolol, which decreased Cdyn and increased RL both, at period B only. Aerosol atenolol ($beta_1$ antagonist) and butoxamine ($beta_2$ antagonist) were administered to principals at period B to determine if the effect of propranolol was due to $beta_1$ and/or $beta_2$ effects. Atenolol and butoxamine increased RL and decreased Cdyn. Therefore, during the acute disease principals have increased sympathetic drive to $beta_{1\&2}$ receptors.

Normal ponies airways were preconstricted with iv histamine before receiving aerosols of either isoproterenol ($beta_{1\&2}$ agonist), clenbuterol ($beta_2$ agonist), or atenolol plus isoproterenol to determine the beta receptor subtype mediating bronchodilation. Clenbuterol and isoproterenol both attenuated the histamine-induced airway obstruction. Atenolol did not block the bronchodilation following isoproterenol administration, which suggests that bronchodilation in response to beta agonists is mediated primarily via $beta_2$ receptors in ponies.

Dedicated to my parents,

Clarence and Mildred Scott,

who taught me that no goal is too great.

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B	baseline
BPM	beats per minute
C_{dyn}	dynamic compliance
EPI	epinephrine
$ED_{65}C_{dyn}$	estimated dose of drug to decrease C_{dyn} to 65% of the baseline value
FEV_1	forced expiratory volume, maximum amount of air that can be exhaled in one second
f	respiratory frequency
heaves	recurrent obstructive pulmonary disease
iv	intravenous
NANC	nonadrenergic noncholinergic nervous system
NE	norepinephrine
PaO_2	arterial oxygen tension
$PaCO_2$	arterial carbon dioxide tension
P/B	post blockade
PBS	phosphate buffered saline
P_{ES}	esophageal pressure
PL	transpulmonary pressure
PERIOD A	principal ponies in clinical remission

PERIOD B	principal ponies during acute airway obstruction
PRINCIPAL	pony with recurrent obstructive pulmonary disease
R_L	pulmonary resistance
$R_{L0.1}$	R_L at 0.1 mg/ml drug dose
S	aerosol saline
SP	substance P
$\dot{V}$	airflow
VIP	vasoactive intestinal peptide
V_E	minute ventilation
VT	tidal volume

INTRODUCTION

In 1968 Szentivanyi postulated the "Beta-adrenergic theory" for the abnormality of bronchial asthma (Szentivanyi 1968). He suggested that in asthmatics the abnormal bronchoconstriction in response to various stimuli could be due to a hypersensitivity of the alpha adrenergic receptor system or to a functional deficiency of the beta receptor system. This would contrast with the normal balance between alpha and beta adrenergic systems which appears to favor a beta response with relaxation of bronchial smooth muscle. Based on this theory, alpha receptor stimulation in excess of beta receptor stimulation has been proposed as a cause of obstructive airway diseases (Nadel et al. 1986, Simonsson et al. 1967, Reed 1974, Szentivanyi 1968). However, it is still not clear whether alpha and beta receptor function is abnormal in asthmatics or in pulmonary diseases of animals.

Because asthma is a common disease, there have been many studies on the human autonomic nervous system, and some of these studies suggest an abnormality in the alpha and beta adrenergic receptors. Barnes and coworkers in a study of human alpha adrenergic receptors using radioligand binding techniques demonstrated that the density of alpha receptors was greater in lungs from patients with airway obstruction than from normals (Barnes et al. 1980a). Alpha receptor responsiveness has been demonstrated in bronchial smooth muscle from humans affected with chronic airway disease but not from normal subjects, which suggests an abnormality in alpha receptors (Kneussl and Richardson 1978). Furthermore, following beta adrenergic blockade and in some cases cholinergic blockade, asthmatics may bronchoconstrict in response to inhaled alpha agonists, while normal subjects are unaffected (Black et al. 1982, Patel and Kerr 1975, Snashall et al. 1978). Asthmatic patients bronchoconstrict in response to beta adrenergic blockade whereas normal individuals have minimal or no bronchoconstriction, again suggesting a difference between normal individual and subjects with airway disease (Richardson and Sterling 1969, Tattersfield et al. 1973). Using radioligand binding techniques, studies with ovalbumin-sensitized guinea pigs, an animal model of airway obstructive disease, demonstrated a relative increase in $alpha_1$ receptor numbers and decreased beta receptors in lung tissue (Barnes et al. 1980).

The pony and horse commonly suffer from an asthma-like recurrent obstructive pulmonary disease (heaves), and I postulated that these animals may have abnormalities in their alpha and/or beta adrenergic systems which contribute to the airway obstruction of heaves. The importance of altered adrenergic receptor activity in airways has not previously been investigated in vivo in airway obstructive diseases of ponies.

Study 1 examines alpha-1 adrenergic pulmonary responses by aerosol administration of an alpha agonist and antagonist to normal and diseased ponies. The second study was designed to determine if beta adrenergic blockade had any effect on airway responsiveness to histamine or baseline pulmonary function in the ponies. The next study examines the effect of aerosol beta adrenergic antagonists on pulmonary function and whether these beta adrenergic responses and those seen in the second study were mediated through beta-1 and/or beta-2 receptors. The fourth and final study used beta adrenergic agonists to determine the bronchodilator role of beta-1 and beta-2 receptors in normal ponies whose airways were constricted with iv histamine.

The review of the literature is presented in 3 parts. Pulmonary innervation is reviewed with the major emphasis on adrenergic receptors. Next, recurrent obstructive pulmonary disease or "heaves" in horses and ponies is reviewed. In the final section, the autonomic nervous system in airway disease and studies of pulmonary responses to adrenergic agents are briefly reviewed. The specific aims and objectives of the protocols are then outlined.

CHAPTER 1

LITERATURE REVIEW

PULMONARY INNERVATION

Introduction

The autonomic nervous system regulates many aspects of airway function including smooth muscle tone, mucus secretion, fluid transport across epithelium, release of mediators from inflammatory cells and blood flow in the bronchial circulation (Nadel and Barnes 1984). The autonomic innervation of airways is through sympathetic, parasympathetic, and nonadrenergic noncholinergic (NANC) nervous systems (Figure 1-1) (Richardson 1979). Autonomic control and innervation of the airways is therefore complex and still in need of intensive study.

Parasympathetic Nervous System

The airways of mammals receive a dense cholinergic innervation (Richardson 1979). Cholinergic efferents arise in the vagal nuclei of the brainstem and pass down the vagus nerve to synapse in ganglia that are situated in the airway wall. Stimulation of preganglionic vagal fibers in airway ganglia activates nicotinic cholinergic receptors on ganglionic neurons.

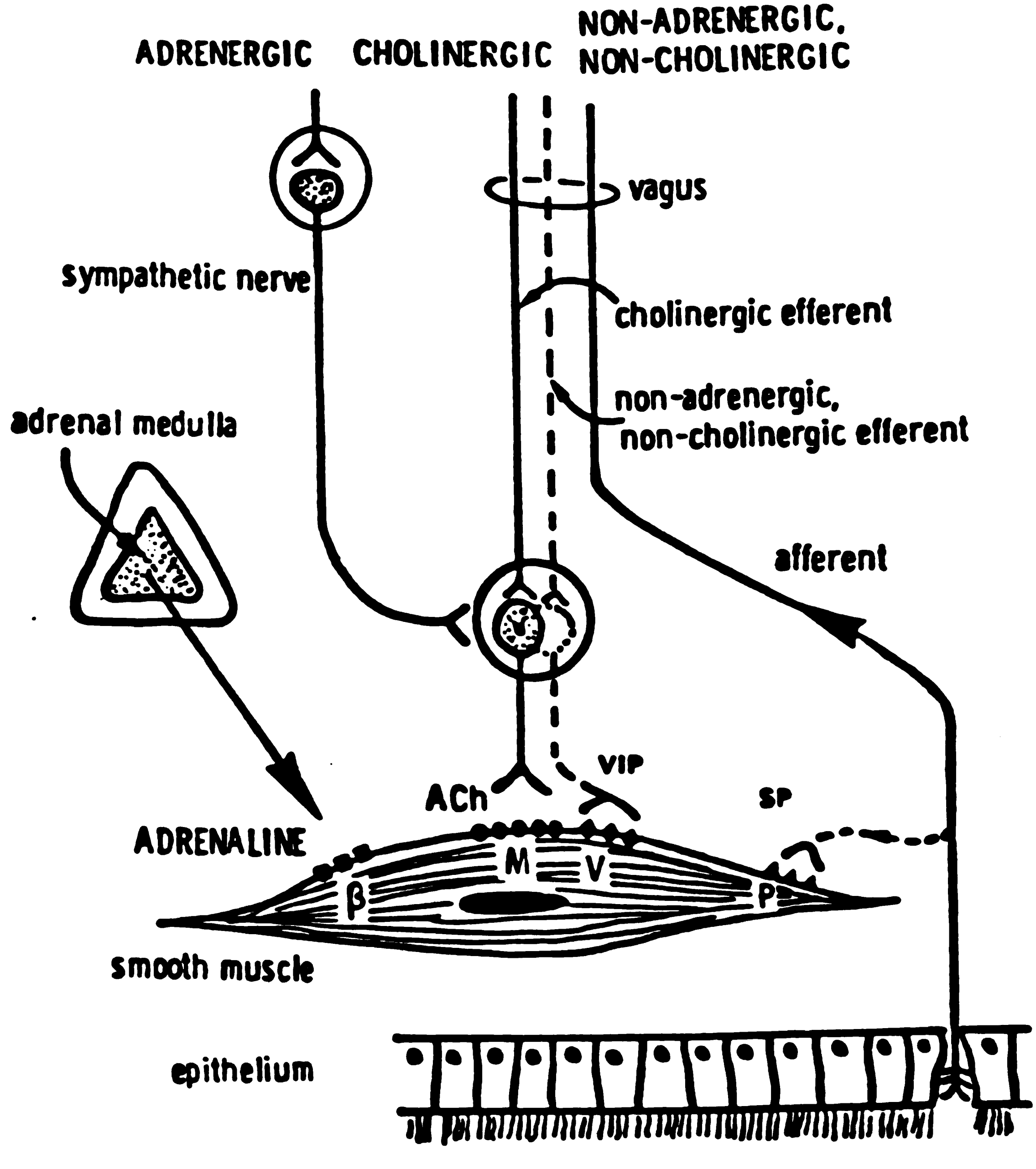

Figure 1-1 Schematic diagram of the innervation to airway smooth muscle. ACh, acetylcholine; VIP, vasoactive intestinal peptide; SP, substance P.

Short postganglionic fibers pass to smooth muscle and other target cells. Efferent stimulation results in release of acetylcholine from the agranular vesicles in cholinergic nerve terminals. Acetylcholine rapidly diffuses to muscarinic cholinergic receptors on the target cell. Subtypes of muscarinic receptors are termed M_1, M_2, M_3, and M_4 (Levine et al. 1985). The receptors on airway smooth muscle cells and tracheal mucosa are of the M_3 subtype (Grandordy et al. 1986, Madison et al. 1987). Prejunctional muscarinic M_2 receptors on cholinergic nerves inhibit acetylcholine release in human bronchi and guinea pig tracheal strips in vitro (Minette and Barnes 1988). In addition, M_1 receptors have been shown to facilitate neurotransmission in airway parasympathetic ganglion in the rabbit (Bloom et al. 1987). Degradation of acetylcholine is by the enzyme acetylcholinesterase, located in the synaptic cleft.

Most species have a certain degree of resting bronchomotor tone which can be abolished by section of the vagus nerve and by atropine, a nonspecific muscarinic receptor antagonist (Nadel 1980). Studies have shown that electrical stimulation of the vagus nerve causes bronchoconstriction (Nadel 1980). This bronchoconstriction is potentiated by acetylcholinesterase inhibitors, and inhibited by muscarinic receptor blockers such as atropine. The effects of vagal stimulation are not equally distributed along airways (Nadel and Barnes 1984). This is most likely due to the distribution and density of the cholinergic innervation which decreases in smaller airways, so that there are few cholinergic fibers in terminal bronchioles, and none seen in the alveolar walls (Barnes 1986a). Furthermore, autoradiographic studies have also shown that the density of muscarinic receptors decreases in smaller airways, so that terminal bronchioles are almost devoid of receptors (Barnes et al. 1983e).

Cholinergic nerve function may be modulated prejunctionally by acetylcholine and other neurotransmitters (Minette and Barnes 1988, Andersson et al. 1986, Danser et al. 1987, Vermiere and Vanhoutte 1979). Presynaptic inhibition of acetylcholine released from vagal nerve endings has been demonstrated for muscarinic M_2 receptors, adrenergic $alpha_2$ and $beta_1$ receptors and prostaglandin E_2 (Minette and Barnes 1988, Andersson et al. 1986, Walters et al. 1984, Danser et al. 1987). Serotonin, substance P, muscarinic M_1 receptors, and prostaglandin F_{2alpha} have been shown to enhance acetylcholine release by prejunctional mechanisms (Hahn and Patil 1972, Sheller et al. 1982, Tanaka and Grunstein 1984).

Sympathetic Nervous System

Innervation

Sympathetic nerves originate from the upper thoracic segments of the spinal cord with postganglionic fibers arising from the cervical and the upper thoracic ganglia. The adrenal medulla, which is itself a ganglion, facilitates sympathetic neural transmission. One preganglionic synapse may activate 20 or more postganglionic neurons as well as adrenal secretion of epinephrine (Innes and Nickerson 1975). This subserves the "flight or fright" response of sympathetic activation. One theory is that focal activation of sympathetic nerves does not occur, and a variety of stimuli elicit this generalized response to all organs (Sands et al. 1985). The postganglionic fibers enter the lung at the hilum accompanying the vagus nerves (Larsell and Dow 1933, Richardson and Sterling 1969). The sympathetic postganglionic neurotransmitter is norepinephrine (NE). Norepinephrine, following action on the postsynaptic muscle membrane, is removed primarily by reuptake into the nerves or degraded by monoamine oxidase (Burnstock 1988). Norepinephrine in plasma is derived almost entirely from overspill of sympathetic nerve activity (Brown et al. 1981). Epinephrine (EPI) is secreted by the adrenal medulla and unlike NE functions as a circulating hormone not a neurotransmitter (Cryer 1980). These catecholamines activate alpha and beta adrenergic receptors on target cells in the airway (Barnes 1984a).

In contrast to the dense parasympathetic nerve supply to airways in all species, sympathetic innervation is generally sparse, and there is much variation among species (Richardson 1979, Doidge and Satchell 1982). For example, adrenergic innervation to human airways is sparse while feline airways receive a rich sympathetic innervation (Doidge and Satchell 1982, Silva and Ross 1974). Histochemical and electron microscopic studies have demonstrated adrenergic innervation of human submucosal glands, bronchial blood vessels, and airway ganglia, but direct adrenergic supply to intrapulmonary airway smooth muscle is very scant or absent (Partanen et al. 1982, Sheppard et al. 1983). There is no evidence for direct innervation of smooth muscle of the small airways, and the distribution of adrenergic innervation to large airway smooth muscle is variable between species (Richardson 1979). Studies in guinea pigs have demonstrated sympathetic nerve fibers to tracheal smooth muscle but not to bronchial smooth muscle (Doidge and Satchell 1982, O'Donnel et al. 1978). While there is no evidence for direct sympathetic nerve supply of intrapulmonary airways of guinea pigs, stimulation of sympathetic outflow to the lungs produces bronchodilation via adrenergic receptors (Ainsworth et al. 1981). In canine airways the amount of bronchodilation from sympathetic stimulation depends on the degree of preexisting vagal tone (Cabezas et al. 1971). This suggests that sympathetic nerves may have a modulatory influence on cholinergic neurotransmission. Norepinephrine inhibits firing of airway ganglion cells in cat and ferret via alpha adrenergic receptors, and studies using canine bronchi have demonstrated a presynaptic $beta_1$ adrenergic inhibition of parasympathetic tone (Danser et al. 1987, Baker et al. 1983).

Beta Adrenergic Receptors

In 1967, Lands and coworkers defined two subtypes of beta adrenergic receptors termed $beta_1$ and $beta_2$ (Lands et al. 1967). The major physiological difference between $beta_1$ and $beta_2$ receptors is their differential sensitivity to norepinephrine (NE). $Beta_1$ adrenergic receptors display approximately equal affinity for EPI and NE, whereas at $beta_2$ receptors EPI is considerably more potent than NE. Therefore, the $beta_2$ receptor has been considered a hormonal receptor, whereas the $beta_1$ receptor is a neuronal receptor (Ariens and Simonis 1983). However $beta_1$ receptors respond to both neuronal and hormonal stimulation and $beta_2$ receptors can be activated by high concentrations of NE (Ariens and Simonis 1983). Both subtypes of beta adrenergic receptors appear to be coupled in a stimulatory fashion to the enzyme adenylate cyclase, which generates cyclic AMP from ATP (Sutherland et al. 1971). Stimulation of this enzyme system, with consequent enhancement of the activity of cyclic AMP-dependent protein kinases represents the biochemical mechanism of action of beta-adrenergic agonists (Glass and Krebs 1980).

Studies using radioligand binding have demonstrated a high density of beta receptors in the lungs of all species examined (Barnes et al. 1980a, Rugg et al. 1978). Beta receptors are found in smooth muscle of all airways from trachea to terminal bronchioles. The density of beta receptors increases with decreasing size of airways in all species studied (Carstairs et al. 1985, Barnes et al. 1983e). When Lands and coworkers subdivided beta adrenergic receptors into $beta_1$ and $beta_2$ they classified airway smooth muscle receptors as $beta_2$ (Lands et al. 1967). Since then both $beta_1$ and $beta_2$ receptors have been demonstrated in airways (Furchgott et al. 1975). Relaxation of tracheal smooth muscle in response to beta adrenergic agonists in some species is intermediate between a $beta_1$ and $beta_2$ mediated response (Furchgott et al. 1975). Relaxation of feline tracheal smooth muscle, which has a dense sympathetic innervation was found to be predominantly mediated by $beta_1$ receptors (O'Donnell and Wanstall 1983, Silva and Ross 1974). In canine tracheal smooth muscle, relaxation in response to exogenous beta agonists is mediated by $beta_2$ receptors, whereas relaxation in response to sympathetic nerve stimulation is mediated by $beta_1$ receptors (Barnes et al. 1983b). These findings are consistent with the theory that $beta_1$ receptors are activated by sympathetic nerves and $beta_2$ receptors are activated by circulating catecholamines (Ariens and Simonis 1983). Human airway smooth muscle appears completely devoid of sympathetic nerves with the inhibitory nerves being entirely nonadrenergic (Richardson 1981, Richardson and Ferguson 1979a, Barnes 1986). Furthermore, relaxation of human airway smooth muscle by catecholamines, including NE is solely mediated by $beta_2$ receptors (Zaagsma et al. 1983). Using receptor binding techniques the ratio of $beta_1$:$beta_2$ receptors in the human lung was found to be approximately 3:1 (Rugg et al. 1978, Engel 1981). Airway beta receptors account for less than 5% of total lung beta receptors with >90% being localized to alveolar walls (Barnes et al. 1982, Carstairs et al. 1984).

Human submucosal glands which receive a sparse adrenergic innervation have beta receptors of which approximately 10% are $beta_1$ receptors (Carstairs et al. 1985, Pack and Richardson 1984, Meyrick and Reid 1970). $Beta_1$ and $beta_2$ receptors stimulate mucus secretion (Phipps et al. 1982). Epithelial and mast cells that are not innervated have only $beta_2$ receptors (Carstairs et al. 1985, Hughes et al. 1983). The high density of beta receptors in the epithelium from large bronchi to terminal bronchioles of human lung exceeds the density of receptors in the corresponding smooth muscle layers (Carstairs et al. 1985). In bovine trachea, the density of beta receptors is two fold higher and the affinity is six fold greater in the epithelial membranes than in the smooth muscle (Agrawal et al. 1987). Similarly, the epithelium of rat bronchioles has a higher density of beta receptors than the smooth muscle (Xue et al. 1983). These data explain the effects of beta adrenergic agonists on mucociliary transport by enhancing fluid transport across the epithelium and by increasing glandular mucus secretion (Mossberg 1979).

Alpha Adrenergic Receptors

Two types of alpha adrenergic receptors have been identified, $alpha_1$ and $alpha_2$ (Langer 1974, Starke et al. 1975). $Alpha_1$ receptors are generally postsynaptic and display high affinity for the $alpha_1$ selective antagonist prazosin. $Alpha_2$ receptors may be either presynaptic where they mediate feedback inhibition of NE release from sympathetic nerve terminals or postsynaptic. $Alpha_2$ receptors have high affinity for the antagonist yohimbine and appear to be coupled in an inhibitory fashion to the enzyme adenylate cyclase, thus regulating the levels of cyclic AMP within the cell (Michel et al. 1982). In contrast, $alpha_1$ adrenergic receptors stimulate the hydrolysis of phosphatidylinositol bisphosphate leading to the generation of two putative second messengers, inositol trisphosphate (IP3) and diacylglycerol (Lefkowitz and Caron 1986). Diacylglycerol is the endogenous activator of the enzyme, protein kinase C, while IP3 appears to mobilize intracellular calcium stores (Lefkowitz and Caron 1986).

There are relatively few alpha adrenergic receptors in the lung (Barnes et al. 1979). In the ferret, $alpha_1$ receptors are sparse in large airways but numerous in small airways with the highest density in smooth muscle of the bronchioles (Barnes et al. 1983e). In canine smooth muscle, radioligand binding studies have demonstrated predominately $alpha_2$ receptors and very few $alpha_1$ receptors (Barnes et al. 1983a). Autoradiographic studies have demonstrated that alpha receptors are localized to serous cells of submucosal glands and alpha agonists facilitate release of histamine from human mast cells (Barnes and Basbaum 1983d, Kaliner et al. 1972). $Alpha_2$ receptors may also be present in airway ganglia, since NE inhibits firing of neurons in airway ganglia, and activation of presynaptic $alpha_2$ receptors may inhibit acetylcholine release from cholinergic nerve endings (Baker et al. 1983, Grundstrom et al. 1981).

Alpha adrenergic receptors which mediate smooth muscle contraction have been demonstrated in many species although alpha adrenergic responses may only be demonstrated following beta adrenergic and cholinergic blockade (Kneussl and Richardson 1978, Simonsson et al. 1972). Human peripheral lung strips contract in response to NE, suggesting that small airways might have $alpha_1$ receptors (Black et al. 1981). Functional studies on the beta receptor blocked guinea pig lung strip preparation showed the NE induced contraction to be mediated entirely by $alpha_1$ receptors (Van der Heijden 1984). In canine tracheal smooth muscle contraction resulted from stimulation of both $alpha_1$ and $alpha_2$ adrenergic receptors on tracheal muscle (Leff and Munoz 1981). Although no alpha adrenergic contraction can be elicited in normal human airways, when airways are diseased or exposed to endotoxin, alpha responses appear, suggesting that disease may activate alpha receptors (Kneussl and Richardson 1978, Simonsson et al. 1972).

Nonadrenergic Noncholinergic Nervous System

In addition to the cholinergic and adrenergic nervous systems in airways there is a third nervous system, the NANC (Barnes 1984, Richardson 1981). In the gastrointestinal tract the NANC nervous system has been recognized for many years (Burnstock 1972). Since the embryological development of the airways is from the foregut, the existence of NANC nerves in the lung is

logical.

Electrical field stimulation of precontracted smooth muscle in the presence of adrenergic and cholinergic blockade in the airways of some species produces relaxation (Cameron et al. 1983, Coburn and Tomita 1973). These data suggest that there is another nervous system other than the cholinergic or adrenergic systems which mediates smooth muscle relaxation of airways. The neurotransmitters in NANC inhibitory nerves are thought to be vasoactive intestinal peptide (VIP) and peptide histidine methionine (PHM) (Said 1982, Palmer et al. 1986). Receptors for VIP are found in the smooth muscle of proximal airways, submucosal glands and airway epithelium (Carstairs and Barnes 1986a). Administration of vasoactive intestinal peptide in isolated human and bovine airways produces smooth muscle relaxation that mimics the effect of NANC stimulation (Cameron et al. 1983, Palmer et al. 1986). In cholinergic nerves, VIP coexists with acetylcholine and reduces both the contractile response to exogenous acetylcholine and to electrical field stimulation (Laitinen et al. 1985). Therefore, VIP may act as a functional antagonist to acetylcholine since the reduction of contractile response occurs without a change in the number or affinity of muscarinic receptors (Palmer 1985, Laitinen et al. 1985). In vitro, PHM is a relaxant of human bronchi of similar potency to VIP (Palmer et al. 1986).

There is evidence in the guinea pig that electrical field stimulation also produces atropine resistant contractions of airways which suggests that a noncholinergic excitatory system exists (Andersson and Grundstrom 1983). Substance P and other tachykinins may be involved in NANC excitatory nerves (Lundberg et al. 1984). Substance P immunoreactivity has been localized to airway nerves of several species, including humans (Lundberg et al. 1984). There is a high density of substance P receptors in airway smooth muscle from trachea to bronchioles, with less density on airway epithelium (Carstairs and Barnes 1986). Infusion of substance P in vivo causes bronchoconstriction and in vitro contracts airway smooth muscle in several species (Karlsson et al. 1984, Andersson and Persson 1977). The gene that codes for substance P also codes for a related peptide, neurokinin A, which also causes bronchoconstriction when administered intravenously to normal subjects (Nawa et al. 1983, Clarke et al. 1987).

RECURRENT OBSTRUCTIVE PULMONARY DISEASE (HEAVES)

Introduction

Horses and ponies commonly suffer from a spontaneously occurring recurrent obstructive pulmonary disease also known colloquially as "heaves" or "broken wind" (Lowell 1964, Thurlbeck and Lowell 1964). Recurrent obstructive pulmonary disease is thought to have been recognized as early as 333 BC by Aristotle, who described the characteristic expiratory effort or "heave" associated with this condition (Smith 1919). Clinical signs of the disease include inspiratory and especially expiratory dyspnea, diffuse wheezing, increased mucus production, coughing, and decreased exercise tolerance (Willoughby and McDonnell 1979, Gillespie and Tyler 1969, McPherson et al. 1978). Recurrent obstructive pulmonary disease is precipitated by exposing susceptible animals to a barn environment and hay (Cook 1976). Normal ponies are not affected by barn exposure and hay feeding (Derksen et al. 1985). Because heaves is a clinical sign indicative of airway obstruction, the etiology of heaves may be multifactorial, including diet and infectious agents, but it is most commonly thought to be an allergic hypersensitivity primarily to thermophilic mold spores found in "moldy" hay and possibly barn dust (Halliwell et al. 1979, McPherson et al. 1979, Gerber 1973). Clinical remission usually occurs when affected animals are put in a pasture and have no contact with the antigen (Derksen et al. 1985).

During acute disease exacerbations affected animals are hypoxemic, have an increased pulmonary resistance (R_L), decreased dynamic compliance (C_{dyn}), prolongation of nitrogen washout, airway hyperresponsiveness, and increased neutrophil numbers in bronchoalveolar lavage fluid (Derksen et al. 1985, Derksen 1985a, Gillespie et al. 1966, Sporri and Denac 1971, Willoughby and McDonnell 1979). Histologically, heaves is characterized by diffuse bronchiolitis, goblet cell metaplasia, airway smooth muscle hypertrophy, bronchiolar epithelial hyperplasia, acinar overinflation, peribronchiolar inflammatory cell infiltration, and excessive mucus and inflammatory cells in small airways (Thurlbeck and Lowell 1964, Wilkie 1982, Nyman et al. 1989, Breeze 1979).

This recurrent obstructive disease of ponies has features in common with human asthma including the chronicity of the condition with intermittent exacerbations, multifactorial etiology, lung lesions such as excessive mucus and inflammatory cells in the airways, natural occurrence of the disease, and response to beta adrenergic therapy (Derksen et al. 1985, Leff 1982, Murphy et al. 1980). One of the underlying factors in heaves therefore could be an imbalance in the autonomic nervous system similar to that suggested for asthmatics, with the cholinergic and/or alpha adrenergic systems predominating over the beta adrenergic system. This will be discussed in the section on the autonomic nervous system and airway disease. While heaves as a disease entity has been known for centuries, very little information is available on the autonomic regulation of horse and pony airways.

Etiology

The possible etiologies of recurrent obstructive pulmonary disease include allergy, diet, and previous infection (Halliwell et al. 1979, McPherson et al. 1979, Gerber 1973). The evidence in support of causes of heaves other than allergy is circumstantial. Three-methylindole (3MI), a derivative of dietary tryptophan that causes acute respiratory distress syndrome in cattle on pasture, has been suggested to play a role in heaves by investigators who induced obstructive lungs disease in horses using 3MI (Derksen et al. 1982, Breeze et al. 1978). However, a natural role of 3MI in heaves seems unlikely since tryptophan is found on pasture where horses with recurrent obstructive pulmonary disease go into remission.

Heaves might occur more frequently in horses following a previous respiratory infection but there is little evidence in support of this theory. Gerber found an increase in the frequency of heaves following an outbreak of equine influenza in 1965 in Switzerland (Gerber 1970).

In 1959, Alexander postulated an allergic pathogenesis for heaves, and in 1964 Lowell succeeded in showing a relationship between acute disease exacerbations and the feeding of hay (Lowell 1964, Alexander 1959). Lowell's study showed that heaves can develop when horses inhale the 'heaves-producing factors' from hay (Lowell 1964). The etiology of heaves is now most commonly thought to be an allergic hypersensitivity to thermophilic mold spores found in hay and organic dust antigens in a barn environment (Halliwell et al. 1979, McPherson et al. 1979, Gerber 1973). The two predominant etiological antigens are thought to be Micropolyspora faeni and Aspergillus fumigatus (Halliwell et al. 1979, McPherson et al. 1979, McPherson and Thomas 1983). Micropolyspora faeni is a thermophilic actinomycete that can cause "farmer's lung" or hypersensitivity pneumonitis in people (Pepys 1969). However, the role of allergy in the pathogenesis of heaves still remains controversial. Lawson et al., examined the sera of normal horses and horses with heaves for precipitins to Micropolyspora faeni and Aspergillus fumigatus (Lawson et al. 1979). Precipitins to both antigens were not restricted to animals with heaves and many animals without detectable precipitins responded with clinical signs similar to heaves after inhalation challenge with these antigens (Lawson et al. 1979). Furthermore, it has been suggested that recurrent obstructive pulmonary disease has an allergic etiology because intradermal skin testing with possible antigens from hay sometimes results in immediate or delayed (4-6 hours) skin reactions but similar skin swelling can occur in normal horses (Breeze 1979). However, Halliwell and coworkers (1979) demonstrated a positive correlation between a 30 minute skin reaction and an immediate reaction to bronchial provocation with mold antigens in horses with heaves.

Whether allergy, diet, or infection causes heaves in horses and ponies has not yet been determined. The possible mechanisms which lie between the cause and the effect of bronchoconstriction and airway hyperreactivity in recurrent obstructive pulmonary disease may include abnormalities in the autonomic nervous system which has had little study in horses.

Pulmonary Function Tests

Amoroso and Sporri in the early 1960's were the first to adapt pulmonary function equipment for use in horses (Amoroso et al. 1962, Sporri and Leeman 1964). Since the horse suffers from a pulmonary disease similar to asthma it was natural for pulmonary function tests of normal and diseased horses to be developed. Pulmonary function tests can be used as an aid in diagnosis of recurrent obstructive pulmonary disease, to follow the progress of the disease, to evaluate the functional changes that occur before and after various drugs are administered, and to select effective therapy for the disease. Before pulmonary function tests were developed, horses with heaves were diagnosed on the basis of clinical signs and history only.

The first lung function measurements developed were of the "work of breathing" evaluated by the maximum change in pleural pressure (PL) measured using an esophageal balloon or a pleural cannula during tidal breathing, and pulmonary gas exchange using the arterial oxygen tension (PaO_2) and $PaCO_2$ (Obel and Schmiterlow 1948, Alexander 1959, Gillespie et al. 1966, Gillespie et al. 1964, Derksen and Robinson 1980). Horses with heaves have a greater change in pleural pressure than normal horses and they are hypoxemic (Gillespie et al. 1966, Gillespie et al. 1964, Muylle and Oyaert 1973, Sasse 1971). Various techniques including whole body plethysmography and pulmonary function computers have been developed for use on horses to also measure air flow, tidal volume, respiratory frequency, dynamic compliance, and pulmonary resistance (Derksen et al. 1985, Gillespie and Tyler 1969, Gillespie et al. 1966, Muylle and Oyaert 1973, Sasse 1971, Beadle 1986, Lekeux 1986, Amoroso et al. 1962, Sporri and Leeman 1964).

One common method used in unanesthetized horses to measure air flow and tidal volume is integrated pneumotachography (Derksen et al. 1985, Gillespie et al. 1966, Muylle and Oyaert 1973, Sasse 1971, Sporri and Leeman 1964, Mauderly 1974, Dewes et al. 1974). The pneumotachograph consists of a resistance such as a mesh screen and a differential pressure transducer to detect the pressure drop across the resistance during air flow (Willoughby and McDonnell 1979). The pressure difference across the resistance is proportional to the flow rate, and conversion of flow to volume is accomplished by electronic integration of the flow signal (Willoughby and McDonnell 1979). The tidal volume and air flow signals can then be displayed on a suitable recorder (Willoughby and McDonnell 1979). The pneumotachograph to be used can be attached to a horse face mask or an endotracheal tube inserted into the airway (Willoughby and McDonnell 1979, Gillespie et al. 1966, Derksen et al. 1985). Recorded values for the change in pleural pressure, tidal volume and flow, can be used to calculate dynamic compliance (C_{dyn}) and pulmonary resistance (R_L). Dynamic compliance is calculated by dividing the tidal volume in liters by the change in transpulmonary pressure (PL) (cm H_2O) between points of zero flow. The two points of zero air flow are at end expiration and end inspiration (Willoughby and McDonnell 1979). Pulmonary resistance is the ratio of the change in PL to the change in flow (liters/sec) at points of equal lung volume (Amdur and Mead 1958, Willoughby and McDonnell 1979). Dynamic compliance is lower and R_L is higher in horses with recurrent obstructive pulmonary disease than in normal horses (Willoughby and McDonnell 1979, Derksen et al. 1985, Armstrong et al. 1986, Broadstone et al. 1988).

While the change in PL is higher in horses with heaves, tidal volume, respiratory frequency, and $PaCO_2$ values are the same as normal horses (Willoughby and McDonnell 1979, Derksen et al. 1985, Armstrong et al. 1986, Broadstone et al. 1988). Pulmonary function measurements such as the maximum volume of air that can be exhaled in one second (FEV_1), performed on asthmatics, can not be used on horses due to a lack of conscious cooperation. Therefore, the pulmonary function measurements discussed above are more practical to use on unsedated horses and ponies.

Airway Hyperresponsiveness

Like the airways of asthmatics, airways of ponies and horses with heaves are hyperresponsive to various stimuli (Armstrong et al. 1986, Derksen et al. 1985, Derksen et al. 1985b, Obel and Schmiterlow 1948). Hyperresponsiveness causes the airways of "heavey" ponies to narrow in response to stimuli that do not affect normal ponies to the same degree. The airways of animals with heaves are hyperresponsive to aerosol and intravenous histamine, aerosol methacholine, aerosol water, and aerosol citric acid (Armstrong et al. 1986, Derksen et al. 1985, Derksen et al. 1985b, Obel and Schmiterlow 1948, Robinson 1989a). Obel and Schmitterlow, in 1948 were the first to demonstrate that horses with heaves have airways hyperresponsive to intravenous histamine, but these investigators did not examine the time course for the hyperresponsiveness (Obel and Schmiterlow 1948). It is now known that ponies with recurrent obstructive pulmonary disease have hyperresponsive airways during acute disease exacerbations but not during clinical remission when their airway responsiveness is similar to normal ponies (Derksen et al. 1985).

Airway responsiveness in ponies is measured using techniques similar to those used to determine airway responsiveness in asthmatics. Increasing concentrations of a drug such as histamine or methacholine are administered to a pony usually by aerosol or sometimes intravenously. Pulmonary function variables such as R_L and C_{dyn} are measured after each dose of the drug. A dose response curve is constructed plotting C_{dyn} and R_L vs. dose of the drug. The dose of drug required to decrease C_{dyn} to 65% of the baseline value ($ED_{65}C_{dyn}$) is calculated by interpolation between points on the dose response curve (Figure 1-2). Therefore, ponies that show a greater decrease in $ED_{65}C_{dyn}$, or increase in R_L at 0.1 mg/ml drug dose ($R_{L0.1}$), when compared to normal subjects or clinical remission values are considered to have hyperresponsive airways. Figure 1-2 demonstrates that ponies with heaves, during acute disease exacerbations, have a leftward shift in the dose response curve when compared to remission values and normal ponies.

The underlying mechanism for airway hyperresponsiveness in ponies is unknown. Derksen and coworkers measured pulmonary function and airway reactivity to aerosol and intravenous histamine in "heavey" and normal ponies in vivo (Derksen et al. 1985, Derksen et al. 1985b). The investigators found that the ponies with recurrent obstructive pulmonary disease were hyperreactive to both aerosol and intravenous histamine during the acute disease but not during remission (Derksen et al. 1985, Derksen et al. 1985b). In addition the control ponies did not have hyperreactive airways at any time period measured (Derksen et al. 1985, Derksen et al. 1985b). The authors concluded that the airway obstruction and reactivity of ponies with heaves is similar to some occupational asthma-like diseases such as airway obstruction resulting from the exposure to Western red cedar dust (Derksen et al. 1985). They also suggest that ponies with heaves may develop an allergy to antigens in a barn environment after many years of exposure to barn dust and hay. However, the mechanism of recurrent obstructive pulmonary disease and airway hyperresponsiveness remains unknown.

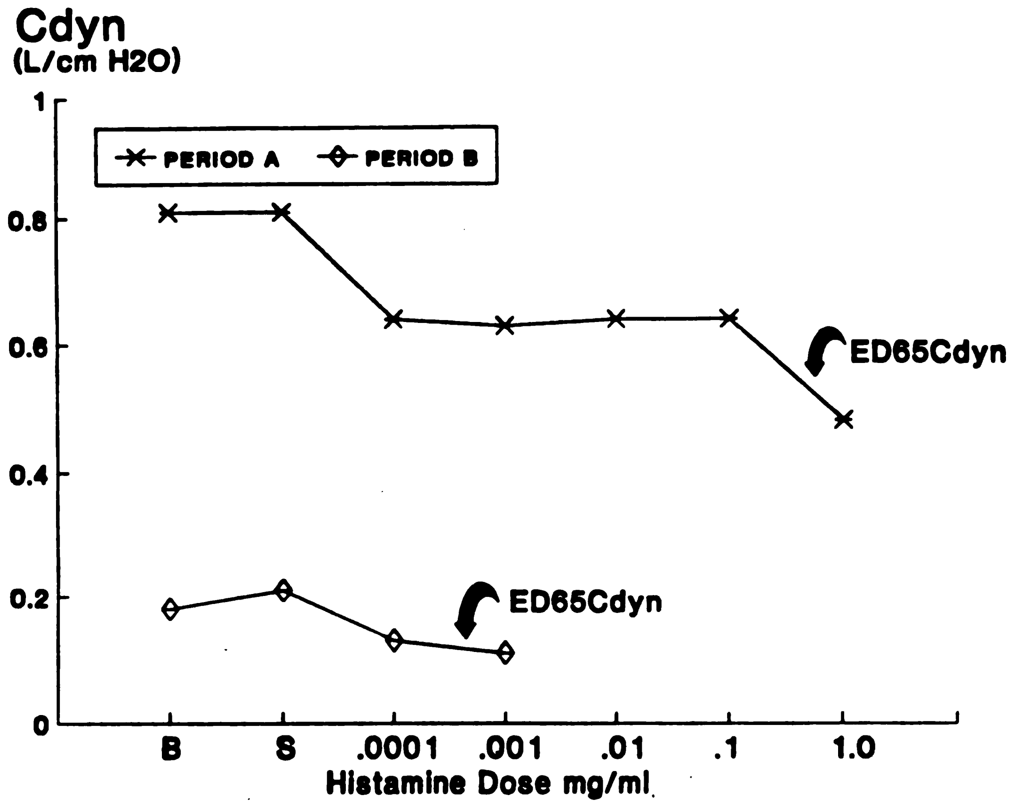

Figure 1-2 Dose response curve showing the effect of aerosol histamine on dynamic compliance (C_{dyn}) at baseline (B), after administration of aerosol saline (S) and after increasing logarithmic doses of histamine (mg/ml) in a pony with recurrent obstructive pulmonary disease (principal) at periods A and B. Arrows indicate the estimated dose of histamine required to decrease C_{dyn} to 65% of baseline value ($ED_{65}C_{dyn}$). Period A, principal pony in clinical remission; Period B, principal pony during acute airway obstruction.

Autonomic Nervous System

The airways of most mammals receive a dense cholinergic innervation and varying degrees of sympathetic and NANC innervation depending on the species (see the pulmonary innervation section of the literature review) (Doidge and Satchell 1982, Richardson 1979, Sheppard et al. 1983, Cameron et al. 1983, Coburn and Tomita 1973, Richardson 1981, Richardson and Ferguson 1979a, Barnes 1986). Histochemical studies outlining the distribution of autonomic nerves to the airways of horses have not been published.

In vitro studies using normal equine airway smooth muscle have demonstrated that the major excitatory innervation to equine airways is cholinergic (Mason et al. 1989, LeBlanc et al. 1989). The cholinergic innervation appears to be most dense in the larger airways of the horse such as tracheal smooth muscle, and the innervation decreases as the size of the airways decrease (Mason et al. 1989, LeBlanc et al. 1989). An in vivo study using the muscarinic receptor antagonist, atropine, is in support of the cholinergic innervation being most dense in the largest airways (Broadstone et al. 1988). When heavey ponies experience an acute disease exacerbation, atropine decreases R_L but does not change C_{dyn} (Broadstone et al. 1988). In contrast, administration of atropine to normal ponies or heavey ponies in remission in vivo does not alter C_{dyn} and R_L or airway responsiveness to histamine which suggests that these animals have little resting bronchomotor tone (Broadstone et al. 1988). This suggests an activation of cholinergic innervation only during an acute disease exacerbation in vivo, and that the distribution of the cholinergic innervation is primarily to the larger airways. At the same time period, during the acute disease state, the airways of ponies with heaves are hyperresponsive to aerosol methacholine, a cholinergic agonist, in vivo but airway smooth muscle is hyporesponsive to acetylcholine in vitro (Armstrong et al. 1986, Broadstone et al. 1989). These data suggest that the hyperresponsivness of airways of ponies with recurrent obstructive pulmonary disease is not due to a primary defect of the airway smooth muscle but to some abnormality extrinsic to the smooth muscle cell (Broadstone et al. 1989).

In vitro studies using electrical field stimulation on precontracted smooth muscle have demonstrated that the sympathetic nervous system innervates the normal horse trachea (Robinson et al. 1989, Olson et al. 1989, Olson et al. 1989a). However, both heavey and normal horses lack sympathetic innervation to the smooth muscle of the third generation bronchi (Robinson et al. 1989).

A study using normal horse tracheal smooth muscle precontracted with histamine and treated with atropine and the beta adrenergic antagonist propranolol, demonstrated inconsistent contractile responses to alpha adrenergic agonists which suggests that alpha receptors may be present in horse airways (LeBlanc et al. 1989). The presence and function of alpha adrenergic receptors in normal horses or in horses with heaves in vivo has yet to be determined.

Beta adrenergic agonists, such as isoproterenol, in vitro induce relaxation of normal and heavey horse tracheal smooth muscle precontracted with histamine which demonstrates the existence of beta adrenergic receptors in horse airways (Olson et al. 1989, Robinson et al. 1989, Hanna and Eyre 1980).

Furthermore, in a study in vitro of normal horse smooth muscle, relaxation of precontracted tissues was elicited by a nonspecific $beta_{1\&2}$ agonist and a $beta_2$ agonist to a similar extent, which leaves the beta receptor subtype function yet to be determined (Olson et al. 1989). In addition, there was no difference in isoproterenol-induced relaxation of normal and heavey horse airway smooth muscle in vitro, which demonstrated the presence of beta receptors in both groups but not the subtypes (Robinson et al. 1989).

Administration of beta agonists to horses and ponies with heaves in vivo can cause bronchodilation (Murphy et al. 1980). In most species this bronchodilation is mediated via $beta_2$ adrenergic receptors (Barnes 1986a). However, intravenous clenbuterol, a $beta_2$ adrenergic agonist, had no effect on baseline pulmonary function measurements and did not prevent the increase in R_L induced by aerosol histamine in normal ponies (Derksen et al. 1987). In vivo studies on ponies and horses have not been performed to determine the subtypes of beta receptors mediating airway relaxation or the role of beta adrenergic receptors in heaves.

A NANC inhibitory innervation has also been demonstrated in vitro in normal horse airways and in airways of horses with recurrent obstructive pulmonary disease (Broadstone et al. 1989a). The NANC inhibitory innervation is absent to the third generation bronchi of horses with heaves when compared to normal horses (Broadstone et al. 1989a). Unfortunately, the importance of these findings to the pathogenesis of heaves and airway hyperresponsiveness has not been determined.

In summary, as determined by studies in vitro, the major excitatory innervation of the horse airway is cholinergic with a possible alpha adrenergic component. The role and function of the alpha adrenergic receptors in vivo have yet to be determined. Airways of horses are relaxed via the beta adrenergic and NANC inhibitory systems with some differences in distribution between normal and heavey horses. The relative importance and possible abnormalities of $beta_1$ and/or $beta_2$ adrenergic receptors in vivo in recurrent obstructive pulmonary disease of horses is still in question and has yet to be determined.

AUTONOMIC NERVOUS SYSTEM IN AIRWAY DISEASE

Introduction

The most common models of airway disease similar to recurrent obstructive pulmonary disease in ponies are asthma in humans, ovalbumin-sensitized guinea pigs (sensitized with ovalbumin then challenged with aerosol ovalbumin), ascaris-sensitized dogs and sheep (challenged with ascaris antigen), and Basenji-Greyhound dogs (naturally hyperresponsive airways). The primary naturally occurring model of heaves is asthma, and therefore most of the data in this section on possible abnormalities in the autonomic nervous system in airways will be from studies on human airways with limited data from animal studies interspersed.

Human asthma is a syndrome in which many stimuli can cause airway smooth muscle contraction, mucus hypersecretion, and regional inflammation of airways (Leff 1982). Similar to heaves in horses, asthma is characterized by recurrent periods of airway obstruction interspersed with periods of clinical remission (Leff 1982, Derksen et al. 1985). There are many precipitating causes for an asthmatic attack including allergen exposure, infection, exercise, psychological stress, and administration of drugs such as beta adrenergic antagonists (Richardson and Sterling 1969, Rubenfeld 1977, Strauss et al. 1977, Empey et al. 1976, Townley et al. 1965, McNeill et al. 1966). Asthmatics respond with excessive airway narrowing following the administration of a variety of stimuli that have little effect on airways of normal people (Moreno et al. 1986). This exaggerated responsiveness, or bronchial hyperreactivity, was first described in 1921 when Alexander and Paddock observed that asthmatics but not normal individuals wheezed when given pilocarpine (Alexander and Paddock 1921). Resembling ponies with recurrent obstructive pulmonary disease, asthmatics have enhanced airway responsiveness to pharmacological and physical agents such as histamine, methacholine, prostaglandins, cold air, sulfur dioxide, and inert dust (Strauss et al. 1977, Nadel et al. 1965, Mathe et al. 1973, Dubois and Dautrebande 1958, Curry 1947, Townley et al. 1965).

Autonomic Imbalance Theory

Several investigators have proposed that an imbalance in autonomic control of airways with the excitatory cholinergic and alpha adrenergic influences predominating over the beta adrenergic inhibitory effects on smooth muscle, might underlie airway obstructive diseases such as asthma (Nadel et al. 1986, Alexander 1921, Simonsson et al. 1967, Reed 1974, Szentivanyi 1968, Barnes 1983). An increased response to cholinergic stimuli can result in airway smooth muscle contraction, enhanced mediator release from mast cells and increased secretion by tracheobronchial glands (Nadel 1980, Barnes 1986a). Furthermore, alpha adrenergic receptor stimulation may also result in release of inflammatory mediators from mast cells, bronchoconstriction and increased mucus secretion (Nadel and Barnes 1984, Barnes 1986a, Barnes and Basbaum 1983d, Kaliner et al. 1972). In addition, failure of the beta adrenergic system or abnormalities in beta receptors could result in the removal of inhibition of mast cell degranulation, impaired airway smooth muscle relaxation, and drier mucus (Phipps et al. 1982, Mossberg 1979, Nadel and Barnes 1984, Barnes 1986a). Therefore increased cholinergic and alpha adrenergic responses along with decreased beta adrenergic responses could underlie obstructive airway disease.

Cholinergic Responses

Parasympathetic nerves provide the primary bronchoconstrictor neural pathway in airways (Nadel and Barnes 1984). It is therefore possible that the increased level of bronchoconstriction and airway hyperresponsiveness characteristic of asthma is a result of overactivity of this system. This increased activity could be a consequence of a direct effect on the vagal nerve supply at one or more locations from the vagal afferents to the preganglionic fibers that emanate from the central nervous system to the postganglionic fibers that arise from the ganglia situated in the airway walls (Nadel and Barnes 1984, Richardson 1979, Boushey 1985). Alternatively, there may be an increase in end organ responsiveness to acetylcholine, either by an increase in muscarinic receptor number and/or affinity or by a postreceptor mechanism

(Barnes 1986a, Mita et al. 1983, McKay and Brooks 1983). Evidence supporting an abnormality in cholinergic responsiveness in airway disease is inconclusive.

Asthmatics are hyperresponsive to cholinergic agonists such as methacholine in vivo which suggests an increased cholinergic responsiveness of airway smooth muscle, but increased airway responsiveness is also seen with other agonists such as histamine and prostaglandins (Boushey et al. 1980, Gross and Skorodin 1984). Furthermore, Roberts and coworkers failed to demonstrate any relationship between methacholine responsiveness of human airways in vivo and in vitro, which suggests that there is not a primary abnormality of the smooth muscle (Roberts et al. 1984). Additionally, in patients with chronic obstructive pulmonary disease, there is also a failure of in vivo methacholine responsiveness to correlate with cholinergic, adrenergic or nonadrenergic responses in vitro (Taylor et al. 1985).

Since data suggest that there is not a primary abnormality of airway smooth muscle, Holtzman and coworkers investigated where methacholine does act to cause bronchoconstriction in asthmatics (Holtzman et al. 1980). The authors investigated the effects on the bronchomotor responses to methacholine, of pretreatment with aerosol hexamethonium (ganglionic blocker) and atropine (postganglionic blocker) (Holtzman et al. 1980). Based on their findings the authors suggested that methacholine acts directly at the smooth muscle muscarinic receptor to cause bronchoconstriction (Holtzman et al. 1980). These data raise the question of whether muscarinic receptors are dysfunctional in airway diseases.

Muscarinic receptor numbers are increased in guinea pigs sensitized with ovalbumin then given ovalbumin aerosol, but the receptor affinity is unchanged (Mita et al. 1983). Furthermore, McKay and Brooks, demonstrated an increased sensitivity of guinea pig airways to carbachol following exposure to toluene diisocyanate and attributed this to an increase in the number or affinity of muscarinic receptors which suggests that muscarinic receptors may be abnormal in some models of airway disease (McKay and Brooks 1983). In contrast, bronchial smooth muscle removed from asthmatics undergoing lung resection showed no greater cholinergic sensitivity to a variety of agonists than normal smooth muscle making it difficult to implicate the cholinergic receptor system directly with the pathogenesis airway disease (Cerrina et al. 1985). Prostaglandins, such as PGD_2 and PGF_{2alpha} released from inflammatory cells, enhance cholinergic responsiveness in guinea pigs, and potentiate the bronchoconstrictor effect of methacholine in asthmatics in vivo, but prostaglandins also enhanced histamine responsiveness in the same subjects, suggesting a postreceptor mechanism rather than a receptor-mediated effect (Orehek et al. 1975, Fuller et al. 1986).

Each part of the vagal reflex arc including the vagal afferents has been examined for direct or inferred evidence of malfunction in asthma (Nadel and Barnes 1986, Woolcock and Permutt 1986). Abnormal sensitization of sensory endings, either due to the effect of inflammatory mediators or to their exposure in damaged epithelium has been suggested as a possible malfunction, but evidence is lacking (Gross 1988).

While anticholinergic drugs are effective therapy for some bronchoconstrictor stimuli in asthma they have minimal effects on exercise, histamine and antigen-induced bronchospasm (Gross and Skorodin 1984, Mann and George 1985). In addition, anticholinergic drugs are generally less effective as bronchodilators in asthma than beta adrenergic agonists, which are presumed to reverse bronchoconstriction irrespective of the contractile stimulus (Gross and Skorodin 1984, Karpel et al. 1986).

Even though there is evidence, as discussed above, showing why cholinergic mechanisms might be enhanced in airway obstruction, it is not conclusive. A defect in the adrenergic system could also be reflected by an increase in cholinergic activity since adrenergic nerves can inhibit acetylcholine release presynaptically either via beta receptors or $alpha_2$ receptors (Andersson et al. 1986, Grundstrom et al. 1981, Danser et al. 1987). Presynaptic modulation of cholinergic nerves suggests that mechanisms other than, or in addition to cholinergic, such as adrenergic, could underlie the bronchoconstriction and hyperresponsiveness of diseased airways.

Beta Adrenergic Responses

Dixon and Ransom in 1912 found that the usual response of airway smooth muscle to electrical stimulation of the thoracic sympathetic nerves is bronchodilation, an effect that is abolished by beta adrenergic antagonists (Cabezas et al. 1971, Dixon and Ransom 1912, Castro de la Mata et al. 1962). However, direct sympathetic innervation of human airway smooth muscle does not appear to be a dominant factor in regulating bronchomotor tone (Barnes

1986a). Stimulation of noninnervated adrenergic receptors is important for regulation of airway smooth muscle as was discussed in the first section of the literature review (Barnes 1986a). Since beta adrenergic receptors are the most important endogenous bronchodilator system in airways, diminished beta receptor function would allow the cholinergic and/or alpha$_1$ adrenergic receptors to predominate and constrict airway smooth muscle and release mast cell mediators (Barnes 1988). The possibility of this imbalance in the adrenergic system underlying the enhanced bronchoconstriction associated with airway obstruction has stimulated intense study by many investigators, as will be discussed below.

Szentivanyi originally proposed the beta adrenergic theory of bronchial asthma, with airway hyperreactivity resulting from an abnormality in the bronchodilatory beta$_2$ receptor system (Szentivanyi 1968). Using two animal models of asthma, he postulated that a partial beta adrenergic blockade may be a predisposing factor in the development of asthma (Szentivanyi 1968). Concomitant with this beta receptor dysfunction, Szentinvanyi postulated an increased number and/or activity of alpha$_1$ adrenergic receptors, which mediate bronchoconstriction (Szentivanyi 1968, Szentivanyi 1979, Szentivanyi 1980). Furthermore, using radioligand binding techniques on human lung membrane fractions, Szentivanyi demonstrated a slight decrease in beta adrenergic receptors and a large increase in the numbers of alpha receptors in asthmatics when compared to control subjects (Szentivanyi 1980).

Two findings dispute Szentivanyi's hypothesis, the first is the fact that beta adrenergic agonists reverse bronchospasm in asthmatic airways which indicates that the beta adrenergic receptor system remains functionally important (Fish and Norman 1986, Barnes 1986a). Secondly, while beta antagonists bronchoconstrict airways of asthmatics with minimal effect on normal patients which suggests a defect of beta receptors, chronic administration of beta blockers to normal individuals does not result in asthma and airway hyperreactivity (Leff 1982, Richardson and Sterling 1969, Tatterfield et al. 1973, Barnes 1986a). Therefore there is considerable debate over the importance of the adrenergic system in bronchial asthma and other airway diseases.

Early studies of beta adrenergic responses examined the effects of beta agonists on leukocyte, cardiovascular, and metabolic responses in asthmatics (Cookson and Reed 1963, Parker and Smith 1973, Kariman 1980, Logsdon et al. 1972). While the beta adrenergic receptor density on circulating white blood cells was reduced in asthmatics and impaired beta agonist activation of adenylate cyclase was demonstrated in lymphocytes in vitro, these effects were similar to those found in asthmatic and normal individuals as a result of therapy with beta agonists (Parker and Smith 1973, Logsdon et al. 1972, Galant et al. 1978, Conolly and Greenacre 1976). For example, exposure of nonasthmatics to beta adrenergic agonists can lead to decreased sensitivity to subsequent adrenergic stimulation (Galant et al. 1980b, Svedmyr 1984). This suggests that the defect in beta receptor function in asthmatics might be explained by tolerance resulting from treatment with beta agonists (Barnes 1986a). Furthermore, studies of beta receptor function on cells and tissues remote from the lungs may not indicate any abnormalities in beta receptor function in the airways. However, studies using smooth muscle isolated from bronchi of unmedicated asthmatics undergoing lung resection have demonstrated reduced beta adrenergic relaxation to beta agonists such as isoproterenol (Cerrina et al. 1985, Cerrina et al. 1986, Paterson et al. 1982). This could be due to a reduction in smooth muscle beta receptors numbers or other defects such as a dysfunction in receptor coupling. Unfortunately, in vitro studies of human asthmatics are rare due to limited availability of unmedicated tissues.

Severely affected asthmatics require a higher dose of inhaled beta agonists for bronchodilation in vivo than normal patients which suggests impaired beta receptor function in asthmatics (Barnes and Pride 1983c). Beta adrenergic antagonists such as inhaled or intravenous propranolol, produce bronchoconstriction in asthmatics but have minimal or no effect on normal patients indicating some difference in beta receptor function between asthmatics and normal individuals (Richardson and Sterling 1969, Tattersfield et al. 1973). Beta blockade also increases responsiveness to methacholine in asthmatics but does not increase bronchial hyperresponsiveness to aerosol histamine in normal subjects (Zaid and Beall 1966). The fact that beta blockade affects asthmatic airways and not normal airways and also increases airway responsiveness suggests that there might be a primary malfunction in the beta adrenergic system in obstructive airways disease.

Bronchoconstriction after beta adrenergic receptor blockade implies tonic bronchodilator tone which, in the absence of a functional sympathetic innervation to bronchial smooth muscle, suggests that circulating catecholamines may be important in regulating airway caliber. Plasma catecholamine levels are the same in stable asthmatics as in normal subjects, however, and there also is no relationship between plasma catecholamine concentration and severity of bronchoconstriction (Barnes et al. 1982a). Circulating catecholamines levels in plasma do not increase in asthmatics during bronchoconstriction induced by antigen, methacholine, hyperventilation, or infused propranolol (Barnes et al. 1981b, Ind et al. 1983, Sands et al. 1985, Ind et al. 1984). In contrast, plasma epinephrine fails to rise in asthmatics with exercise-induced bronchoconstriction on exercise, compared with normal individuals, although the response to more severe exercise is normal (Barnes et al. 1981b, Larsson et al. 1982). These data suggest that there may be a defect in initiating epinephrine secretion in exercise-induced asthma but not in other types of asthma.

In addition to studies of human airways, various animal models of airway obstructive disease are also used for studying receptors and smooth muscle function (Nadel and Barnes 1984). In the Basenji greyhound, a canine model with inherent bronchial hyperresponsiveness, beta blockade significantly enhanced bronchial responsiveness to citric acid, but not to methacholine (Hirshman et al. 1981). This suggests that a partial blockade of beta receptors could result in airway hyperresponsiveness to nonspecific stimuli such as citric acid. In this same animal model of asthma, impaired leukocyte beta receptor function was found to be due to a defective nucleotide response (Peters et al. 1981).

Studies in guinea pigs sensitized with ovalbumin and challenged with aerosol ovalbumin, another animal model of obstructive airway disease, demonstrated a 20% reduction in pulmonary beta receptor numbers but no change in beta agonist induced adenylate cyclase activation when compared to normal guinea pigs (Barnes et al. 1980). These data suggest that while the beta adrenergic postreceptor mechanisms may be normal, the number and/or affinity of the receptors is impaired (Barnes et al. 1980). The data from these two animal models therefore suggest that there may be a defect in beta receptors that needs to be investigated. In Ascaris-sensitized dogs however, a reduced concentration of cyclic AMP was found in airway smooth muscle, suggesting decreased activity of beta receptors, but there was no impairment in smooth muscle relaxation to isoproterenol, which shows that no clear picture of beta receptor function exists, reemphasizing the need for further study (Rinard et al. 1979).

Grandordy et al., in a study using bovine tracheal smooth muscle, demonstrated that cholinergic stimulation reduced beta adrenergic receptor density, and uncoupled beta receptors without a decrease in beta receptor function (Grandordy et al. 1987b). However the author suggests that prolonged cholinergic stimulation might result in a functional beta adrenergic receptor defect making this a potentially important mechanism for beta receptor dysfunction in constricted airways (Grandordy et al. 1987b).

The inflammatory mediator, PAF, administered by aerosol to guinea pigs increases bronchial responsiveness to contractile agents while reducing responsiveness to isoproterenol in vivo but the density of beta receptors in vitro is not decreased (Barnes et al. 1987b). These data provide yet another possible mechanism of beta receptor dysfunction while raising more questions than it answers about beta adrenergic receptors and airway disease. Investigations of beta adrenergic receptor function have yielded variable results and leave the question of whether there is a defect in beta receptor function in obstructive airway disease yet to be answered. Additionally, beta receptor function has not been examined in horses and ponies with recurrent obstructive pulmonary disease.

Alpha Adrenergic Responses

Szentivanyi postulated that along with a defect in beta receptor function in asthma there could also be increased alpha adrenergic receptors which mediate bronchoconstriction (Szentivanyi 1968, Szentivanyi 1979, Szentivanyi 1980). There are some studies that support this theory and demonstrate increased alpha receptor responses in asthma; they will be discussed below.

Airways of normal individuals do not constrict in response to alpha adrenergic agonists, but when airways are diseased or exposed to endotoxin, alpha-mediated contraction appears (Simonsson et al. 1972, Kneussl and Richardson 1978). Simonsson demonstrated that human bronchi were unresponsive to the $alpha_1$ agonist, phenylephrine, but contracted in response to phenylephrine following incubation with bacterial endotoxin (Simonsson et al. 1972). Another study reported that an alpha agonist contracted airways obtained from autopsies of patients who had severe respiratory disorders but was without effect on tissues obtained from control subjects (Kneussl and

Richardson 1978). The previous two studies suggest that while alpha adrenergic responses are not evident in airways of normal individuals, they are enhanced in some airway diseases and therefore could be a potentially important mechanism underlying airway obstruction. Additionally, studies using dogs demonstrated that histamine or serotonin pretreatment of smooth muscle unmasks alpha receptor-mediated contraction (Kneussl and Richardson 1978, Barnes et al. 1983f, Brown 1983).

Barnes et al., demonstrated that patients with chronic airway obstruction have a higher density of lung $alpha_1$ receptors than normal humans, which also supports the idea of increased $alpha_1$ receptors in airway disease (Barnes et al. 1980a). Furthermore, the same investigator, using radioligand binding techniques in ovalbumin sensitized guinea pigs, demonstrated a relative increase in $alpha_1$ receptor density (Barnes et al. 1980). In addition to increased alpha receptor numbers as in the previous studies, one study in the canine trachea showed enhanced alpha-mediated contraction that appeared to involve postreceptor mechanisms (Brown et al. 1983). What the postreceptor mechanism is, or if it is important in airway disease has not yet been determined.

Following beta adrenergic blockade, and in some cases cholinergic blockade, asthmatics may bronchoconstrict in response to inhaled alpha agonists, such as phenylephrine, while normal subjects are unaffected (Black et al. 1982, Patel and Kerr 1973, Snashall et al. 1978). In addition, the bronchoconstrictor effect of the alpha agonist methoxamine in asthmatic subjects is inhibited by the selective $alpha_1$ antagonist prazosin, suggesting that this effect is due to $alpha_1$ receptors and that these receptors may be contributing to asthma (Black et al. 1984). However, another study could not demonstrate a bronchoconstrictor effect of alpha agonists in asthmatic subjects which adds to the controversy of the importance of $alpha_1$ receptors in airway obstruction (Thomson et al. 1982).

Enhanced alpha adrenergic responses are not limited to airways since administration locally of the $alpha_1$ agonist phenylephrine to atopic asthmatics demonstrated an enhanced pupillary and cutaneous vascular responsiveness (Henderson et al. 1979). This study suggests that alpha receptor numbers may be increased, or the receptor mechanism changed throughout an asthmatic's system and not just in the airways.

Many studies support the theory that $alpha_1$ adrenergic responses may be increased in asthmatics but to prove that alpha mechanisms are important in asthma depends upon demonstration of the beneficial effects on alpha adrenergic blockers (Barnes et al. 1980a, Kneussl and Richardson 1978, Reed 1974, Szentivanyi 1968). The nonspecific alpha adrenergic blockers thymoxamine, indoramin, and phentolamine inhibit bronchoconstriction induced by histamine, allergen and exercise and also cause bronchodilation in asthmatics (Beil and De Koch 1978, Bianco et al. 1974, Bianco et al. 1972, Kerr et al. 1970, Patel and Kerr 1973, Prime et al. 1972, Campbell 1982). These drugs lack specificity however, and their protective effects may be due to other pharmacological actions such as direct effects on smooth muscle and antihistamine activity (Nadel and Barnes 1984). Prazosin, a specific $alpha_1$ antagonist, has a small protective effect against exercise-induced bronchoconstriction when administered by aerosol suggesting a role for $alpha_1$

receptors in this disease process (Barnes et al. 1981a). However, inhaled prazosin has no bronchodilator effect in chronic stable asthmatics and no effect on histamine-induced bronchoconstiction (Barnes et al. 1981a, Barnes et al. 1981). The role of alpha adrenergic receptors in the pathogenesis of airway obstructive diseases therefore remains controversial and in need of further study in a naturally occurring airway disease such as heaves in ponies.

SPECIFIC AIMS

Several studies of adrenergic receptors in animal models of airway disease and asthma have suggested that there may be altered adrenergic function in obstructive airway disease. The pony and horse suffer from a naturally occurring obstructive pulmonary disease, heaves, which is similar to asthma. Since the importance of altered adrenergic receptor activity in airways of ponies with heaves has not been investigated in vivo, it is the purpose of this thesis to determine if abnormalities in the $alpha_1$ and beta adrenergic systems are involved in the airway obstruction seen with heaves. To accomplish this aim, normal ponies and ponies with recurrent obstructive pulmonary disease were studied in vivo. Experiments were undertaken to:

1. Define the presence and function of $alpha_1$ adrenergic receptors in normal and heavey ponies by measurements of airway responsiveness to an aerosol $alpha_1$ agonist, phenylephrine.

2. Determine the specificity of the response to phenylephrine by use of aerosol prazosin, an $alpha_1$ adrenergic antagonist.

3. Determine the role of $alpha_1$ receptors in airway obstruction by administration of aerosol prazosin to ponies with heaves.

4. Determine the role of beta adrenergic receptors in airway responsiveness to aerosol histamine using the nonspecific antagonist, propranolol, administered intravenously.

5. Determine the role of beta adrenergic receptors in recurrent obstructive pulmonary disease in ponies using aerosol and intravenous propranolol.

6. Determine if the effect of nonspecific beta blockade on airway function in heavey ponies is due to either $beta_1$ and/or $beta_2$ adrenergic receptors using the $beta_1$ blocker, atenolol, and the $beta_2$ blocker, butoxamine.

7. Determine if the bronchodilation seen following administration of beta agonists is due to activation of $beta_1$ and/or $beta_2$ adrenergic receptors using aerosol administration of the nonspecific beta agonist, isoproterenol, the $beta_2$ agonist, clenbuterol, and atenolol ($beta_1$ blocker).

MATERIALS AND METHODS

Introduction

The materials and methods used in all protocols will be described in this section, and the specific experimental designs included in each protocol will be described in the separate chapters. Ponies with a history of recurrent airway obstruction (principals) were matched for age and gender with control ponies with no history of the disease. The pairs of ponies were transported together, housed together, fed the same ration and studied on the same day. Measurements were made when the principal ponies were in clinical remission (period A) and during an acute attack of airway obstruction precipitated by housing principals in a barn and feeding "moldy" hay (period B). Prior to making the pulmonary function measurements at period A the principal ponies were kept on pasture for at least two weeks and had a diet supplemented with a complete pelleted feed as necessary. Principal ponies were considered to have "heaves" when the change in transpulmonary pressure during a tidal volume was greater than 15 cm H_2O and the PaO_2 was less than 75 torr. Lung function and airway responsiveness were measured before and after treatment with adrenergic agents.

Pulmonary function measurements

The ponies were studied unanesthetized standing in stocks. All animals had chronic tracheostomas and carotid arteries relocated to a subcutaneous site. Figure 1-3 shows a schematic of the instrumentation of a pony before each experiment. A 20mm ID cuffed endotracheal tube (45cm length) was introduced in the trachea via the tracheostoma. A pneumotachograph (Fleisch no. 4, Dynasciences, Blue Bell, PA) and associated pressure transducer (Validyne DP 45-22, Northridge, CA) was attached to the endotracheal tube. The pneumotachograph transducer system produced a signal proportional to flow that was electronically integrated to give tidal volume (VT). This system was calibrated before each protocol by forcing known volumes of air through the pneumotachograph using a 2-liter syringe (Super Syringe, Hamilton Syringe, Warminster, PA).

An esophageal balloon (10cm length, 3.5cm perimeter, 0.06cm wall thickness) was sealed over the distal end of a polypropylene catheter (3mm ID, 4.4mm OD, 140cm length) that had spirally arranged holes in the part covered by the balloon (Derksen and Robinson 1980). The distance from the nares of the ponies to the midthoracic portion of the esophagus was visually approximated and marked on the esophageal balloon catheter. The esophageal balloon was passed via the nares into the midthoracic region of the esophagus. Balloon volume was adjusted to contain 0.5ml of air. The balloon was attached to a pressure transducer (Model PM131, Statham, Gould, Inc., Cleveland, Ohio) calibrated to 5 cm H_2O by using a manometer, before each experiment. The pressure transducer was then attached to the stocks. Transpulmonary pressure (PL) was defined as the pressure difference between atmospheric and esophageal pressure (Pes). Transpulmonary pressure, VT, and flow ($\dot{V}$) were recorded on light-sensitive paper (VR12, Electronics for Medicine, White Plains, NY). From these traces, respiratory rate (f) was calculated. Dynamic compliance (C_{dyn}) was calculated by dividing VT by the difference in PL between points of zero flow. Pulmonary resistance (R_L) was calculated at 60% VT using the isovolume method of Amdur and Mead (Amdur and Mead 1958). To prevent phase differences between pressures and flows, frequency responses of catheter systems were matched to 10Hz.

Arterial oxygen tension (PaO_2), CO_2 tension ($PaCO_2$), and pH were measured using a blood gas analyzer which was calibrated daily from known standards, and every 30 minutes with an internal automatic calibration system (Model ABL3, Radiometer, Copenhagen, Denmark). Heart rate was measured throughout some studies using a heart rate computer which was taped to the ponies chest (Model HR7A, Equine Biomechanics and Exercise Physiology, Inc., Unionville, PA).

Airway Responsiveness Measurements

Delivery of aerosol agonists was accomplished by attaching a 2-way non-rebreathing valve to the endotracheal tube. The valve was connected to an ultrasonic nebulizer (Model 65, DeVilbis, Somerset, PA). The output of the nebulizer averages 0.11 ml/2-liter breath and delivers a particle size of 0.5-3.0 micrometers. The ponies were allowed to breathe aerosol spontaneously for two minutes. The nebulizer was disconnected from the endotracheal tube, and the pneumotachograph was attached for measurement of lung function. Transpulmonary pressure, VT and $\dot{V}$ were recorded for two minutes after each aerosol challenge. Dynamic compliance and R_L were averaged over this time period. Exactly 4 minutes after the end of the first challenge, another aerosol challenge was begun. The sequence of challenges was saline followed by increasing logarithmic doses of agonist. Dose response curves of C_{dyn} and R_L were plotted as a function of agonist dose. The dose of agonist required to decrease C_{dyn} to 65% of the saline or baseline value ($ED_{65}C_{dyn}$) was calculated by interpolation between points on the dose response curve which is demonstrated in Figure 1-2. The change in R_L from baseline values to 0.1 mg/ml agonist dose (delta R_L0.1) was also calculated for determinations of airway responsiveness.

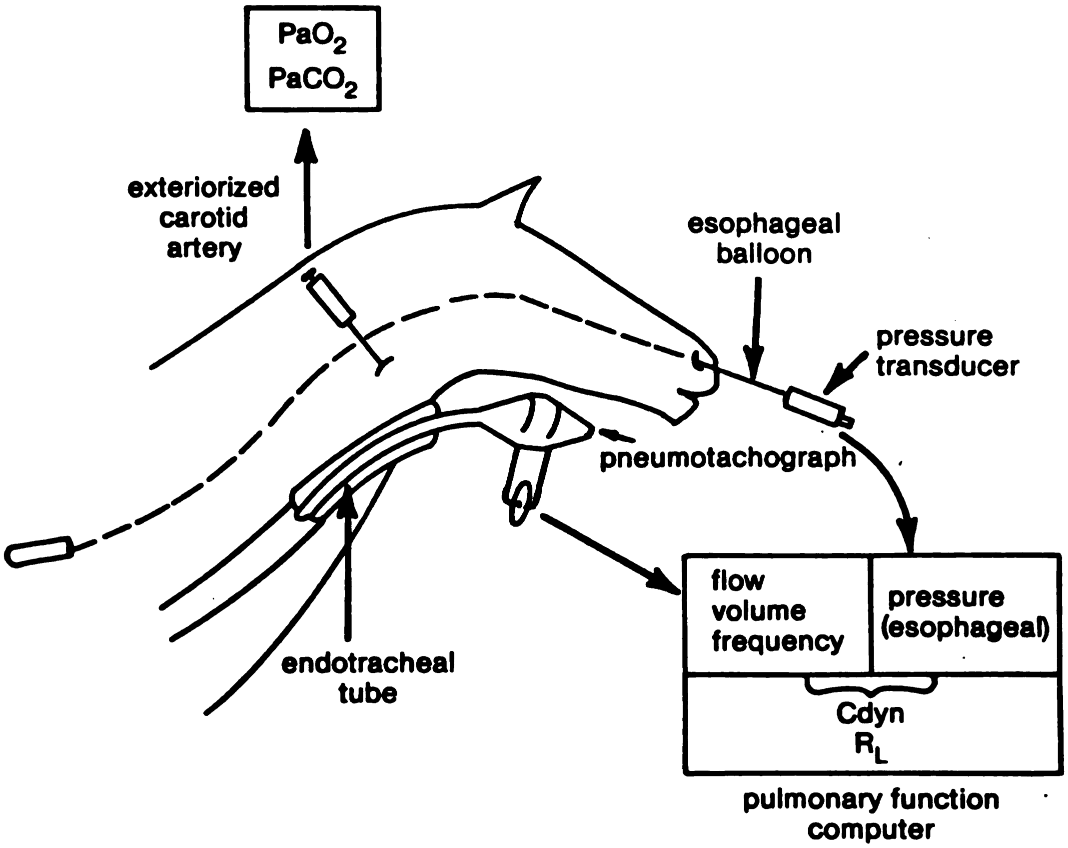

Figure 1-3 Diagram of the equipment used to measure pulmonary function variables in ponies. PaO_2, arterial oxygen tension; $PaCO_2$, arterial carbon dioxide tension; C_{dyn}, dynamic compliance; R_L, pulmonary resistance.

CHAPTER 2

$Alpha_1$ Adrenergic Induced Airway Obstruction in Ponies with Recurrent Pulmonary Disease

Introduction

Alpha adrenergic receptors which may mediate bronchoconstriction have been demonstrated in many species (Kneussl and Richardson 1978, Simonsson et al. 1972). Alpha receptor stimulation in excess of beta receptor stimulation has been proposed as a cause of asthma and bronchial hyperresponsiveness (Barnes et al. 1980, Szentivanyi 1968). Following beta adrenergic blockade and in some cases cholinergic blockade, asthmatics may bronchoconstrict in response to inhaled alpha agonists, while normal subjects are unaffected (Black et al. 1982, Patel and Kerr 1973, Snashall et al. 1978). Alpha receptor responsiveness has been demonstrated in bronchial smooth muscle from humans affected with chronic airway disease but not from normal subjects, and increased pupillary and vascular alpha receptor responsiveness has been demonstrated in allergic asthmatics (Henderson et al. 1979, Kneussl and Richardson 1978). In a study of human alpha adrenergic receptors using radioligand binding techniques the density of alpha receptors was greater in lungs from patients with airway obstruction than from normals (Barnes et al. 1980). Furthermore, some alpha adrenergic antagonists have been shown to prevent bronchoconstriction induced by allergen challenge, histamine and exercise in asthmatics (Beil and De Koch 1978, Bianco et al. 1974, Bianco et al. 1972, Kerr 1970, Patel and Kerr 1975).

In the first section of this study, I examined the response of normal ponies and ponies with recurrent airway obstruction to the aerosol alpha adrenergic agonist, phenylephrine. The ponies were pretreated with atropine and propranolol to decrease cholinergic and beta adrenergic receptor influences, respectively. In the second section of the study, the principal ponies were also pretreated with the specific alpha-1 receptor antagonist prazosin to determine if the response to phenylephrine was an alpha-1 receptor mediated event. Finally, I determined if prazosin is a bronchodilator when administered to the principal ponies during an acute disease exacerbation.

Experimental Design

Five mixed breed ponies with a history of recurrent airway obstruction (principals) were matched for age and gender with a control group of five ponies with no history of disease. Measurements were made with principal ponies in clinical remission (period A) and during an acute attack of airway obstruction precipitated by housing principal and control ponies in a barn (period B). In the first section of this study, lung function and airway responsiveness to the aerosol alpha adrenergic agonist, phenylephrine were measured before and after treatment with atropine and propranolol. Ponies were given a bolus of atropine (0.02 mg/kg) intravenously followed by a continuous infusion (0.0013 mg/kg/min) which was started 15 minutes after the initial bolus (Broadstone et al. 1988). Broadstone and coworkers demonstrated that this dose of atropine shifted the dose response curve to methacholine at least two logarithmic doses in ponies (Broadstone et al. 1988). Propranolol (2.5 mg/kg) was administered intravenously immediately followed by a continuous infusion (0.02 mg/kg/min). This dose of propranolol was determined to be an adequate beta adrenergic blocking dose in another study (See Chapter 3). The sequence of aerosol challenges was phosphate buffered saline (PBS) and phenylephrine in PBS, at increasing logarithmic doses (from 2.08 X 10^{-3} mg/ml to 2.08 X 10^{2} mg/ml). In the second section of the study, the principal ponies at period A were also pretreated with the specific $alpha_1$ receptor antagonist prazosin to determine if the response to phenylephrine was an $alpha_1$ mediated event. Aerosol prazosin was administered until a dosage of 0.11 mg/kg had been nebulized. The ponies then received atropine and propranolol iv followed by aerosol phenylephrine. The prazosin blocking dose, which is ten time the reported human dose (Barnes et al. 1981a), was determined in pilot studies as the amount needed to lower the ponies' blood pressure without causing collapse. Finally, in part 3, I determined if prazosin is a bronchodilator when administered to the principal ponies during an acute disease exacerbation. Lung function was measured before and after aerosol prazosin, and following iv atropine. Pulmonary function and airway responsiveness measurements were performed as described in the materials and methods section above.

Statistical analysis

Statistical analysis was performed using randomized blocked and split plot factorial analysis of variance (Steel and Torrie 1960). When F values were significant at $p < 0.05$, means were compared using Tukey's procedure (Steel and Torrie 1960). Log $ED_{65}C_{dyn}$ comparisons before and after prazosin pretreatment were made using a paired Student t-test at $p < 0.05$ (Steel and Torrie 1960).

Results

The principal ponies during acute obstructive disease (period B) developed hypoxemia (Table 2-1), and had a significant decrease in C_{dyn} and increase in R_L when compared to period A and the control group (Figure 2-1). There were no significant differences in VT, f or $PaCO_2$ between the two groups at either time period (Table 2-1).

The effect of the combined blockade with propranolol and atropine on lung function in both groups is shown in Figure 2-1. Combined blockade had no effect on baseline R_L and C_{dyn} in control ponies at periods A and B or in the principal ponies at period A. However, in the principal ponies at period B, blockade significantly increased C_{dyn} and decreased R_L.

TABLE 2-1 Baseline pulmonary function variables prior to phenylephrine treatment in principal and control ponies at periods A and B. Values are baseline means ± SEM. * Significant difference from period A. + Significant difference between principal and control groups. Period A, principal ponies in remission; period B, principal ponies during airway obstruction.

	Measurement Period	
	A	B
	Control Ponies	
PaO_2, torr	92.4 ± 2.5	97.1 ± 2.6
$PaCO_2$, torr	37.5 ± .8	34.8 ± 1.9
Tidal volume, liters	1.31 ± .09	1.27 ± .12
Frequency, min^{-1}	39.5 ± 5.8	38.6 ± 5.3
	Principal Ponies	
PaO_2, torr	88.2 ± 1.1	68.8±3.8*+
$PaCO_2$, torr	38.3 ± 1.3	40.1 ± 1.7
Tidal volume, liters	1.41 ± .13	1.82 ± .14
Frequency, min^{-1}	37.3 ± 6.2	26.6 ± 3.3

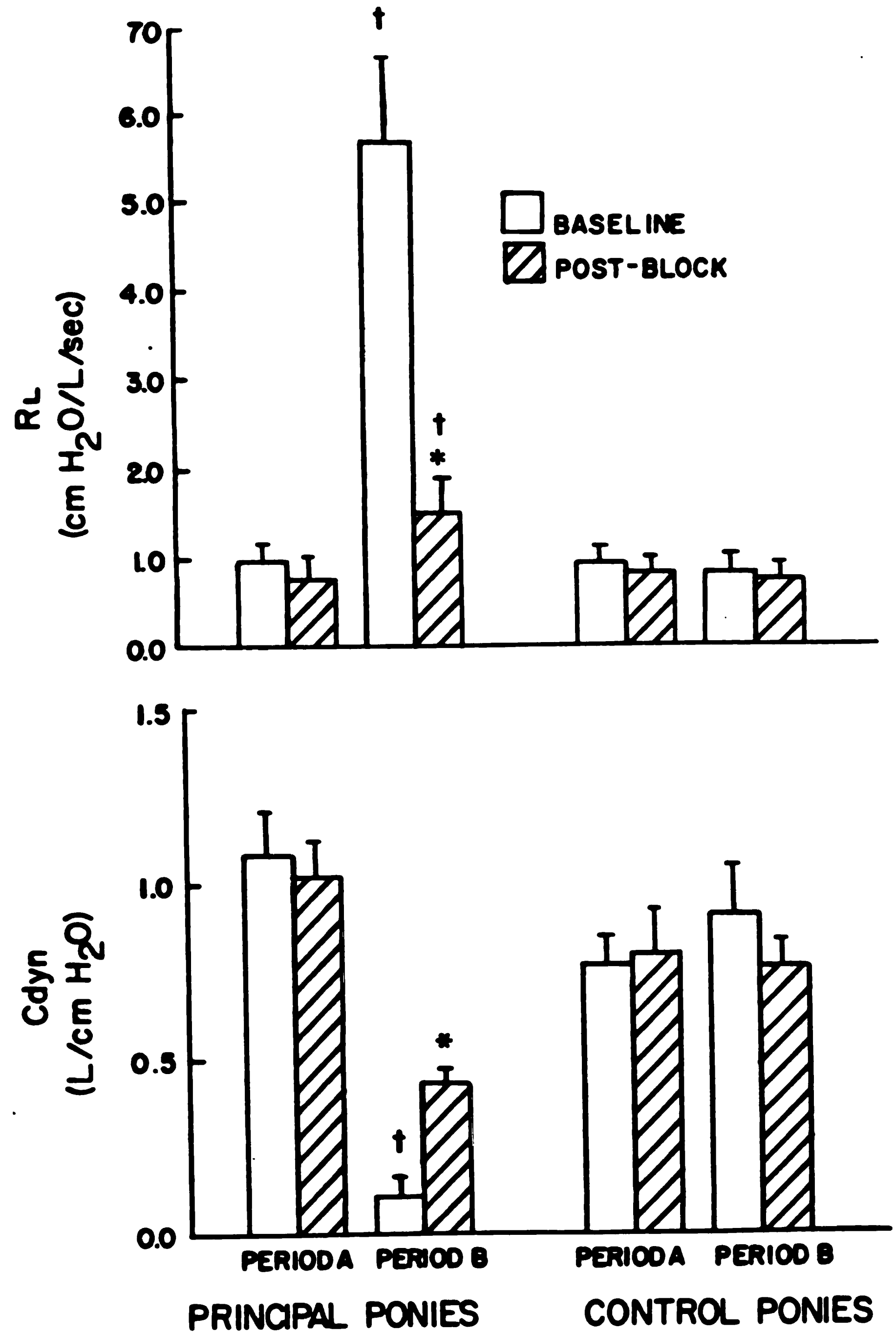

Figure 2-1 Dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) at baseline and after blockade with iv atropine (.02 mg/kg) and iv propranolol (2.5 mg/kg) in 5 principal and 5 control ponies during periods A and B. Period A, principal ponies in clinical remission; Period B, principal ponies during acute airway obstruction. * Significant difference from baseline values.
+ Significant difference from control ponies and period A.

Administration of phenylephrine had no effect on the control group at either time period A or B (Figures 2-2 and 2-3). In the principal ponies, phenylephrine caused a dose-dependent decrease in C_{dyn} (Figure 2-2) and increase in R_L (Figure 2-3) which was similar at both time periods.

When the alpha$_1$ antagonist, prazosin, was added to the combined blockade in the principals at period A, the phenylephrine, C_{dyn} dose response curves were shifted to the right (Figure 2-4). Prazosin also attenuated the increase in R_L seen at the highest dose of phenylephrine (Figure 2-4). In one pony, aerosol prazosin totally blocked the increased R_L and decreased C_{dyn} seen with the phenylephrine dose response. Prazosin administration significantly increased phenylephrine log $ED_{65}C_{dyn}$ (Figure 2-5).

Prazosin administration in the principal ponies at period B had no effect on baseline pulmonary mechanics (Figure 2-6). Atropine iv following aerosol prazosin, significantly increased C_{dyn} and decreased R_L (Figure 2-6). However, prazosin/atropine blockade pulmonary function values were not different from the atropine/ propranolol blockade values (Figure 2-6).

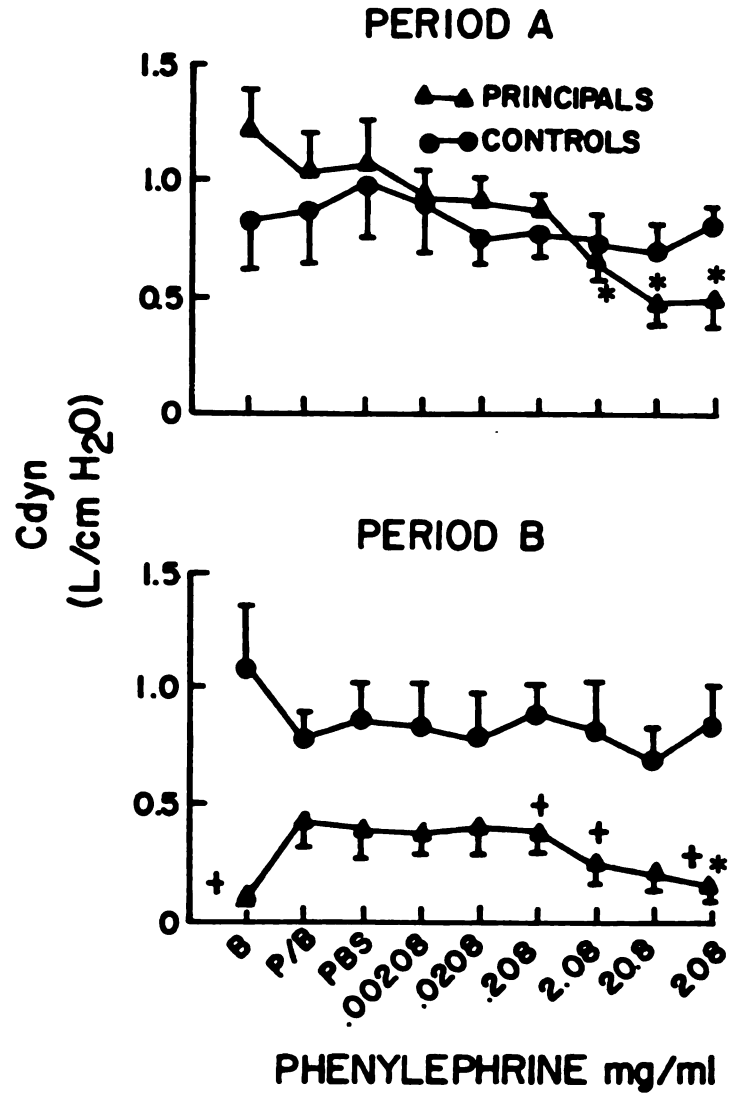

Figure 2-2 Dose response curve showing the effect of aerosol phenylephrine on dynamic compliance (C_{dyn}) in 5 principal and 5 control ponies during periods A and B. B, baseline; P/B, post-iv atropine (.02 mg/kg)/iv propranolol (2.5 mg/kg) blockade; PBS, aerosol phosphate buffered saline; period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction. * Significant difference from baseline values. + Significant difference from control ponies.

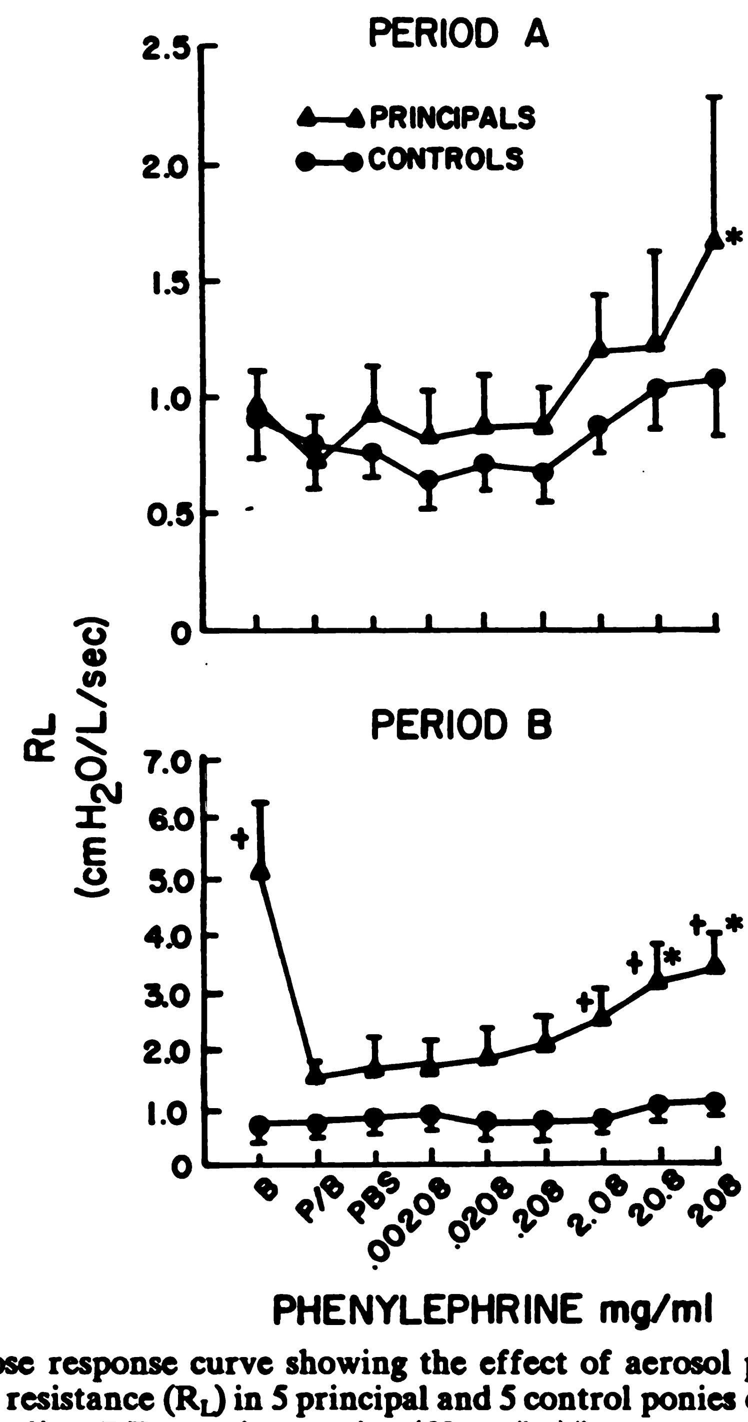

Figure 2-3 Dose response curve showing the effect of aerosol phenylephrine on pulmonary resistance (R_L) in 5 principal and 5 control ponies during periods A and B. B, baseline; P/B, post-iv atropine (.02 mg/kg)/iv propranolol (2.5 mg/kg) blockade; PBS, aerosol phosphate buffered saline; period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction. * Significant difference from baseline values. + Significant difference from control ponies.

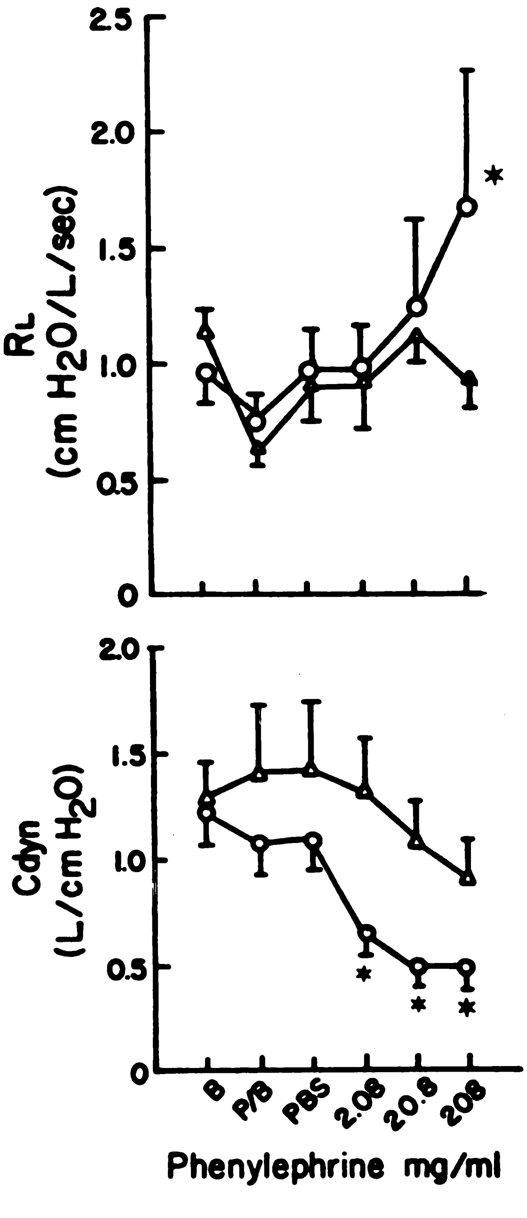

Figure 2-4 Dose response curve showing the effect of aerosol phenylephrine (open circles) and aerosol phenylephrine/aerosol prazosin (.11 mg/kg) (open triangles) on dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) in 5 principal ponies during period A (clinical remission). B, baseline; P/B, post-iv atropine(.02 mg/kg)/iv propranolol (2.5 mg/kg) blockade; PBS, aerosol phosphate buffered saline. * Significant difference from baseline values.

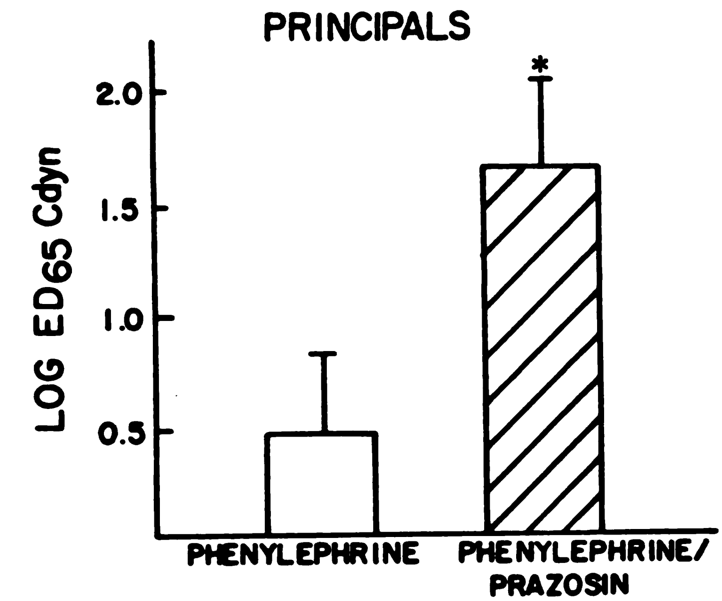

Figure 2-5 The dose of aerosol phenylephrine needed to decrease dynamic compliance (C_{dyn}) to 65% of post-iv atropine (.02 mg/kg)/iv propranolol (2.5 mg/kg) blockade value (log $ED_{65}C_{dyn}$) for aerosol phenylephrine alone, and aerosol phenylephrine plus aerosol prazosin (.11 mg/kg) in 5 principal ponies during period A (clinical remission). * Significant difference from phenylephrine alone.

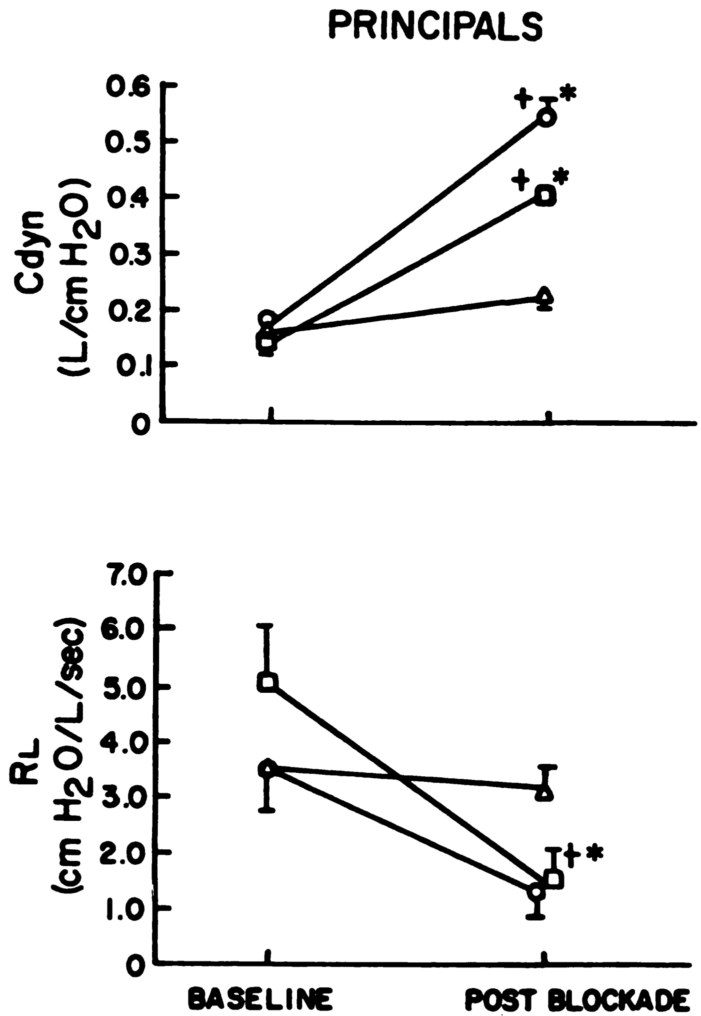

Figure 2-6 Dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) at baseline and after blockade with aerosol prazosin (.11 mg/kg) (open triangles), aerosol prazosin (.11 mg/kg)/iv atropine (.02 mg/kg) (open circles), and iv atropine (.02 mg/kg)/iv propranolol (2.5 mg/kg) (open squares) in 5 principal ponies during period B (airway obstruction). * Significant difference from baseline values. + Significant difference from post-prazosin.

Discussion

Consistent with previous reports, the principal ponies in this study at period B developed airway obstruction manifested by a decreased PaO_2 and C_{dyn} and increased R_L (Armstrong et al. 1986, Broadstone et al. 1988, Derksen et al. 1985). Control ponies housed in the identical environment did not develop changes in lung function. Principal ponies, whether in remission or during acute disease exacerbations, demonstrated responsiveness to the alpha agonist, phenylephrine, when compared to control animals. Furthermore, the shift in the dose response curve to the right following prazosin administration demonstrated that this response was partially an $alpha_1$ adrenergic mediated event. Similarly, studies with asthmatics have demonstrated bronchoconstriction following alpha agonist administration, and increased pulmonary $alpha_1$ receptor density has been demonstrated using radioligand binding in ovalbumin sensitized guinea pigs and also in humans with obstructive lung diseases (Barnes et al. 1980, Barnes et al. 1980a, Patel and Kerr 1975).

In principal ponies at period B, beta adrenergic blockade with iv propranolol increased R_L without a concomitant decrease in C_{dyn} (See Chapter 3). Therefore, the decrease in R_L that was seen in this study with the combined blockade of propranolol and atropine was due to the effect of atropine. This suggests that in the principal ponies during acute disease exacerbations a large proportion of the initially high R_L is due to increased parasympathetic activity and/or hyperresponsiveness to acetylcholine (Armstrong et al. 1986). This, however, is not the only contributing mechanism since post blockade R_L in the principal ponies remained significantly higher at period B than at period A (Figure 2-1). When prazosin was added to the atropine blockade, there was no further decrease in R_L (Figure 2-5). This suggests that mechanisms other than parasympathetic and $alpha_1$ adrenergic activity are contributing to the increase in R_L at period B. The high R_L could be due to mucus plugging, edema and/or an alteration in other neural controls. Similarly, C_{dyn} did not return to a baseline remission value in the principal group even with prazosin added to the atropine blockade, again indicating additional mechanisms other than parasympathetic and $alpha_1$ adrenergic contributing to the airway obstruction of heaves.

Previous studies in ponies with heaves have shown that principal animals have airways hyperresponsive to histamine and methacholine at period B but not at period A (Armstrong et al. 1986, Derksen et al. 1985). The results of the aerosol phenylephrine challenge revealed a difference in alpha receptor responsiveness between the principal and control groups at both time periods. This suggests the two populations of ponies could be distinguished by their alpha receptor responsiveness whether principals were in remission or not. These findings are consistent with other studies where asthmatics bronchoconstrict following alpha agonist administration while normal patients do not (Black et al. 1982, Patel and Kerr 1973, Snashall et al. 1978). The significant decrease in C_{dyn} with the highest dose of phenylephrine in the principal group at both time periods suggests increased alpha receptor activity in the peripheral airways. This is consistent with studies in other species using radioligand binding techniques, demonstrating the highest density of $alpha_1$ receptors located peripherally (Barnes et al. 1983e, Barnes et al. 1983a).

The modest but significant increase in R_L in the principal group following phenylephrine administration may be due to either an increased alpha receptor activity in more centrally located airways, or is a result of diffuse bronchoconstriction in smaller airways. Studies in other species have demonstrated few $alpha_1$ receptors in the central airways which suggests that the increased R_L seen in this study at the highest phenylephrine dose is most likely due to increased alpha receptor activity in the periphery (Barnes et al. 1983e, Barnes et al. 1983a).

Prazosin, a specific $alpha_1$ adrenergic antagonist, shifted the phenylephrine dose response curve to the right with a significant increase in log $ED_{65}C_{dyn}$ in the principal group. This indicates that the airway obstruction seen with aerosol phenylephrine was partially an $alpha_1$ receptor mediated event. Studies using canine airway smooth muscle have demonstrated both $alpha_1$ and alpha-2 adrenergic mediated contraction (Leff and Munoz 1981). It is possible that the increased alpha adrenergic responses in the principal ponies may also be due to alpha-2 receptors, but phenylephrine is primarily an $alpha_1$ agonist which is blocked selectively by prazosin (Cambridge et al. 1977). Aerosol prazosin, when added to the atropine blockade did not significantly change C_{dyn} and R_L. This suggests that the $alpha_1$ adrenergic system has a minimal role in heaves. These findings are consistent with studies where inhaled prazosin had no bronchodilator effect in asthmatics and no effect on histamine-induced bronchoconstriction (Barnes et al. 1981, Barnes et al. 1981a). Some alpha adrenergic antagonists have been shown to prevent bronchoconstriction induced by allergen challenge, histamine, and exercise in asthmatics (Beil and De Koch 1978, Bianco et al. 1974, Bianco et al. 1972, Kerr et al. 1970, Patel and Kerr 1975). Other studies have demonstrated that prazosin partially protects against exercise-induced bronchoconstriction (Barnes et al. 1981a). My study did not determine whether prazosin would attenuate histamine or methacholine-induced bronchoconstriction in ponies. It is possible that the alpha adrenergic system could have a role in the hyperresponsiveness to these agents in principal ponies during an acute disease exacerbation.

In summary, I demonstrated a difference in $alpha_1$ receptor responsiveness between ponies with recurrent obstructive lung disease and normal animals. These findings suggest either an increased density and/or activity of alpha receptors in heavey ponies. The amount that the $alpha_1$ adrenergic system contributes to the bronchoconstriction seen in heaves is probably minimal since prazosin did not significantly increase C_{dyn} or decrease R_L in the principal group at period B.

CHAPTER 3

Beta Adrenergic Blockade with Intravenous Propranolol in Ponies with Recurrent Obstructive Pulmonary Disease

Introduction

Obstructive airway diseases (asthma) and airway hyperresponsiveness were proposed by Szentinvanyi to be caused by abnormalities in the beta adrenergic system (Szentivanyi 1968). Asthmatic patients bronchoconstrict in response to beta adrenergic blockade whereas normal individuals have minimal or no bronchoconstriction (McNeil 1964, Richardson and Sterling 1969). Studies of alpha and beta adrenergic receptors using radioligand binding techniques in lung tissue from an animal model of chronic asthma have demonstrated increased alpha and fewer beta receptors when compared to controls (Barnes et al. 1980). To better understand the possible role of the beta adrenergic system in ponies with heaves, I tested the effect of beta adrenergic blockade with intravenous (iv) propranolol on baseline pulmonary mechanics and airway responsiveness to aerosol histamine.

Experimental Design

I examined the effect of beta adrenergic blockade with propranolol on airway responsiveness to aerosol histamine in 6 ponies with recurrent airway obstruction and 6 age- and gender-matched controls. Measurements were made with principal ponies in clinical remission (period A) and during an acute attack of airway obstruction (period B). Lung function and airway reactivity to aerosol histamine were measured before and after the beta adrenergic blockade with propranolol. The sequence of challenges was saline and histamine diphosphate in saline at increasing log doses (.0001, 0.001, 0.01, 0.1, 1.0, 3.0, 10.0, 30.0 and 100.0 mg/ml). Aerosol challenge was stopped when C_{dyn} decreased to approximately 50% of the baseline value obtained before i.v. administration of sterile water or propranolol. The ponies were given either a 20 ml intravenous sterile water bolus or propranolol (2.5 mg/kg) as a bolus followed by a continuous infusion of 20 ug/kg/min. Adequacy of the beta blockade was confirmed at the end of each experiment by the lack of an increase in heart rate following a bolus iv infusion of 0.2 mg isoproterenol. The dosage rate of isoproterenol used was ten times the amount needed to double a pony's heart rate as determined by pilot studies. Pulmonary function and airway responsiveness measurements were performed as described in the materials and methods sections above.

Statistical Analysis

Statistical analysis was performed using a split plot factorial analysis of variance (Steel and Torrie 1960). When F values were significant at $P < 0.05$, means were compared using Tukey's procedure (Steel and Torrie 1960).

Results

Baseline lung function data of both groups of ponies at periods A and B are shown in Table 3-1 and Figures 3-1, 3-2, 3-3, and 3-4. There were no differences between principal and control ponies in PaCO2, PaO2, f, or VT at period A or in PaCO2, f or VT at period B. However, PaO_2 decreased significantly in the principal group at period B. Baseline R_L and C_{dyn} of the control ponies were unchanged between periods A and B. At period A, R_L and C_{dyn} of the principal ponies did not differ from values measured in the control group. However, in the principal ponies at period B there was a significant increase in R_L and decrease in C_{dyn} when compared to period A. These parameters were also significantly different from the control group at periods A and B.

TABLE 3-1 Baseline pulmonary function variables prior to sterile water or iv propranolol treatment in principal and control ponies at periods A and B. Values are baseline means ± SEM. * Significant difference from period A. + Significant difference between principal and control groups. Period A, principal ponies in remission; period B, principal ponies during airway obstruction.

	Measurement Period A		Measurement Period B	
	Water	Propranolol	Water	Propranolol
		Control ponies		
PaO_2, torr	96.5 ±2.6	99.0 ±4.0	96.9 ±2.5	97.5 ±2.7
$PaCO_2$, torr	35.4 ±1.1	35.8 ±1.7	36.0 ±0.9	37.7 ±0.7
Tidal volume, liters	1.35 ±0.11	1.33 ±0.26	1.37 ±0.12	1.37 ±0.12
Frequency, min^{-1}	25.0 ±3.0	24.0 ±3.0	26.0 ±3.0	26.0 ±3.0
		Principal ponies		
PaO2, torr	95.8 ±3.4	96.1 ±1.4	63.9*+ ±1.7	68.7*+ ±1.7
PaCO2, torr	39.8 ±1.0	38.8 ±1.1	43.5 ±1.2	42.0 ±1.4
Tidal volume, liters	1.73 ±0.16	1.87 ±0.22	1.91 ±0.24	1.87 ±0.17
Frequency, min^{-1}	22.0 ±4.0	30.0 ±4.0	24.0 ±2.0	22.0 ±2.0

The effect of beta adrenergic blockade on lung function in both groups is shown in Figures 3-1, 3-2, 3-3, and 3-4. Propranolol did not change C_{dyn} (Figures 3-1 and 3-2) or R_L (Figures 3-3 and 3-4) in either group of ponies at period A or in the control group at period B. Propranolol treatment also did not significantly change C_{dyn} in the principal group at period B (Figure 3-2). However, beta blockade significantly increased R_L (6.80 $\pm$ .67 to 9.46 $\pm$ .77 cmH2O/L/sec) in the principal group at period B (Figure 3-4). Figure 3-5 shows the change in R_L (delta R_L) from baseline values to after treatment with either sterile water or propranolol in principal ponies at measurement periods A and B. The effect of propranolol on C_{dyn} and R_L in the principal ponies was very consistent; propranolol had no effect on C_{dyn} and R_L in any pony at period A and no effect on C_{dyn} in any pony at period B, yet propranolol increased R_L in every pony at period B (Figure 3-6).

Although log ED_{65}Cdyn (Figure 3-7) and delta R_L0.1 (Figure 3-8) did not change significantly in the principal group between periods A and B, delta R_L0.1 at period B was significantly greater in the principals than in the controls, indicating a difference in airway responsiveness to histamine between the two groups. Dose response curves to histamine at periods A and B after sterile water or propranolol treatment are shown in Figures 3-9, 3-10, 3-11, and 3-12. The magnitude of change in R_L throughout the histamine dose response (Figures 3-11 and 3-12) was variable between ponies, but R_L increased in most animals with increasing histamine doses.

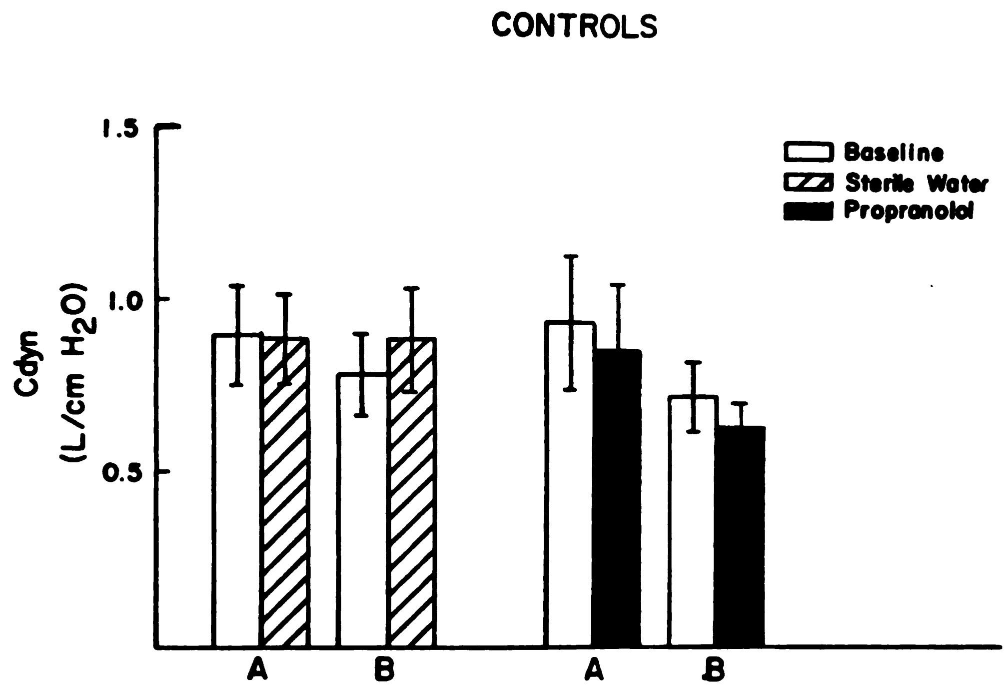

Figure 3-1 Dynamic compliance (C_{dyn}) at baseline and after treatment with iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) in 6 control ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

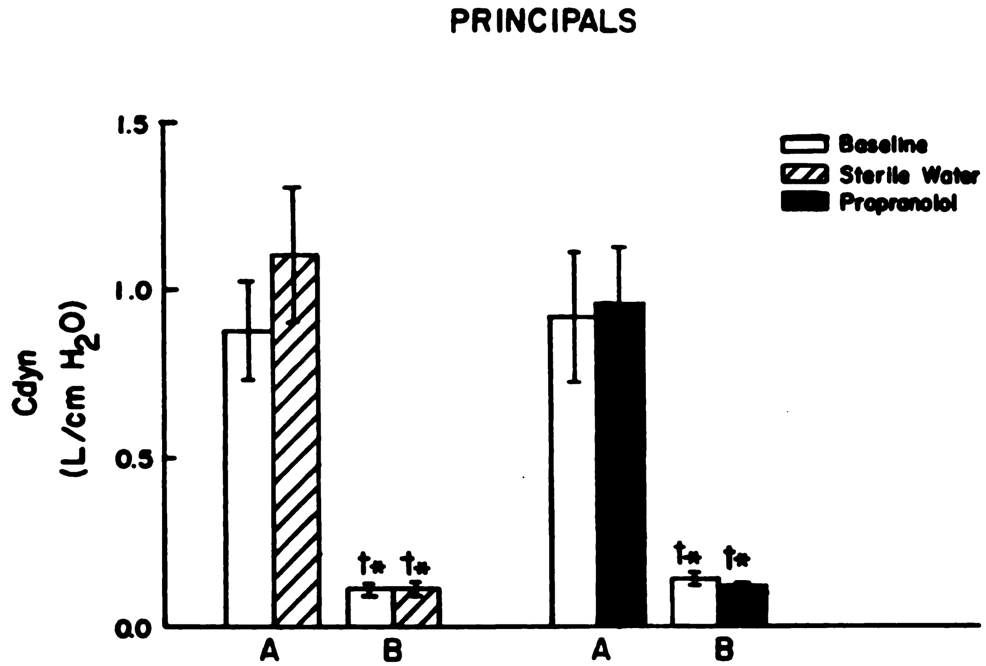

Figure 3-2 Dynamic compliance (C_{dyn}) at baseline and after treatment with iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) in 6 principal ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction. * Significant difference from period A. + Significant difference between principal and control groups.

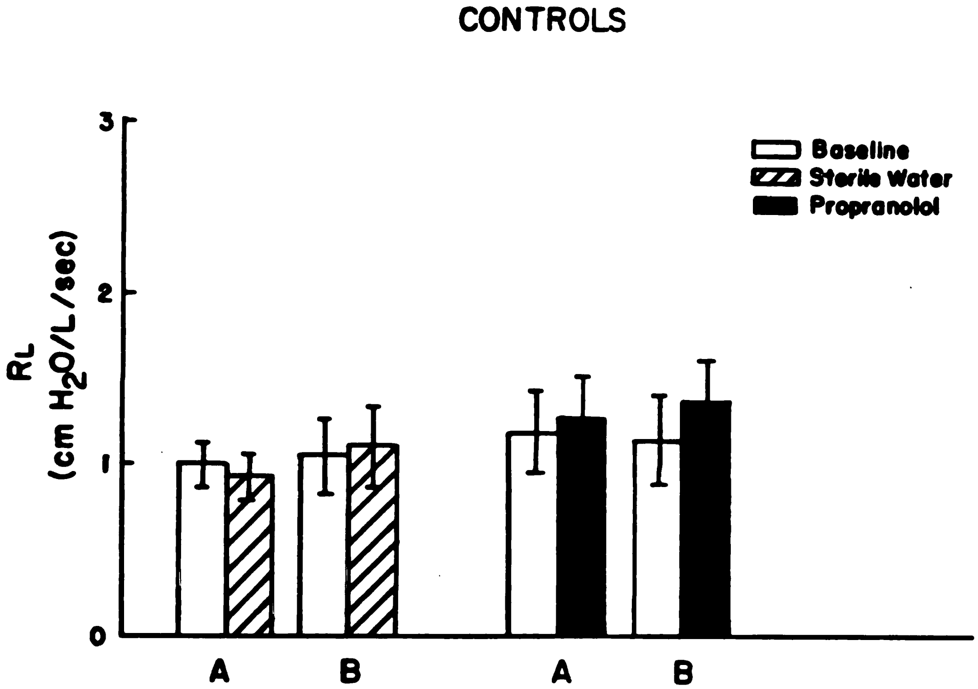

Figure 3-3 Pulmonary resistance (R_L) measured at baseline and after treatment with iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) in 6 control ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

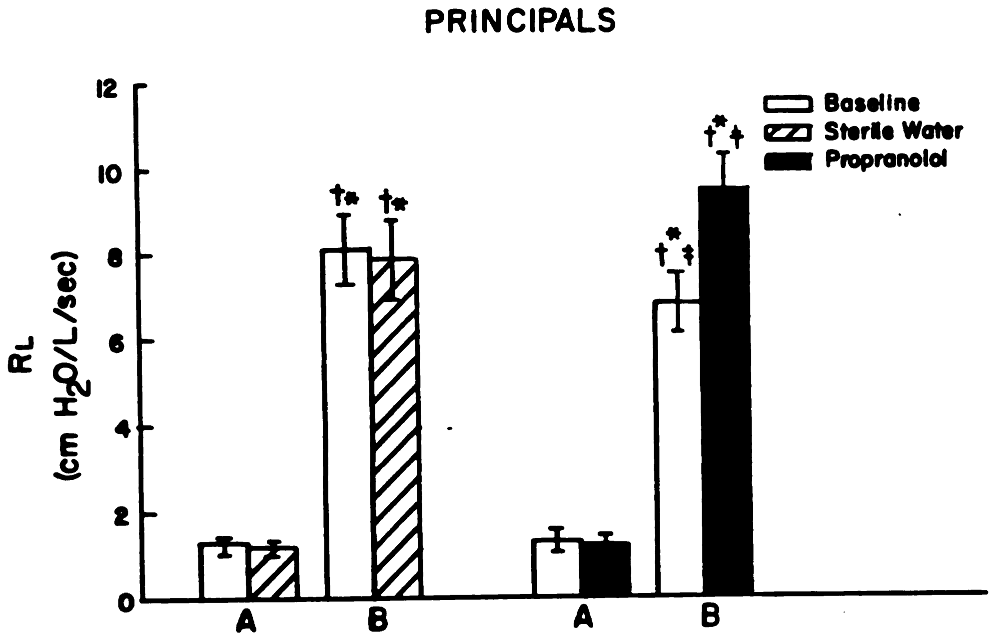

Figure 3-4 Pulmonary resistance (R_L) at baseline and after treatment with iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) in 6 principal ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction. * Significant difference from period A. + Significant difference between principal and control groups. ‡ Significant difference between propranolol and baseline values.

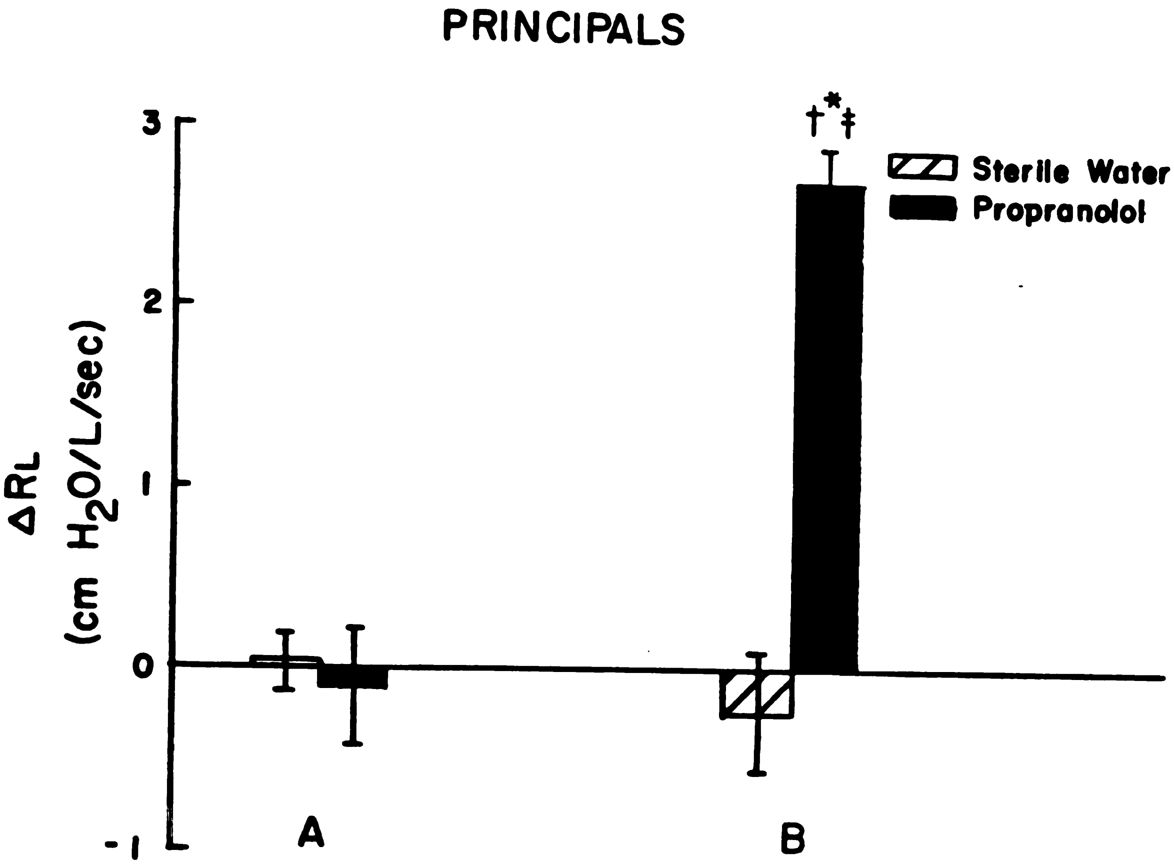

Figure 3-5 The change in pulmonary resistance (delta R_L) between baseline and iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) administration in 6 principal ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.
* Significant difference from period A. + Significant difference between principal and control groups. ‡ Significant difference between propranolol and sterile water values.

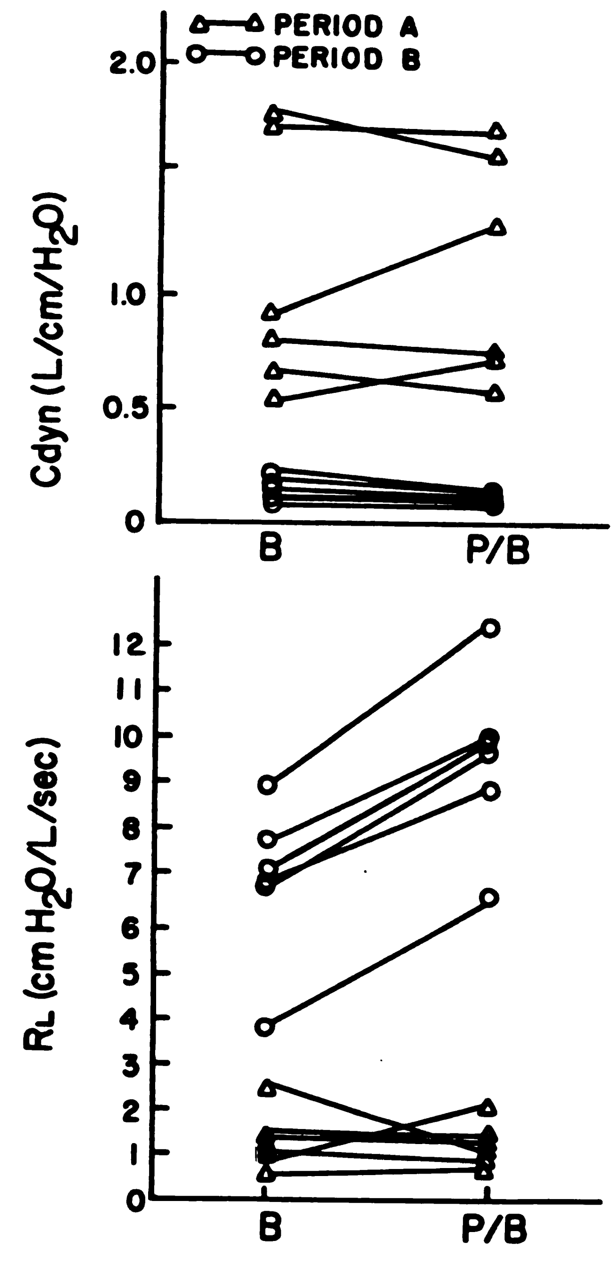

Figure 3-6 Dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) at baseline (B) and post-iv propranolol (2.5 mg/kg) blockade (P/B) in 6 principal ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

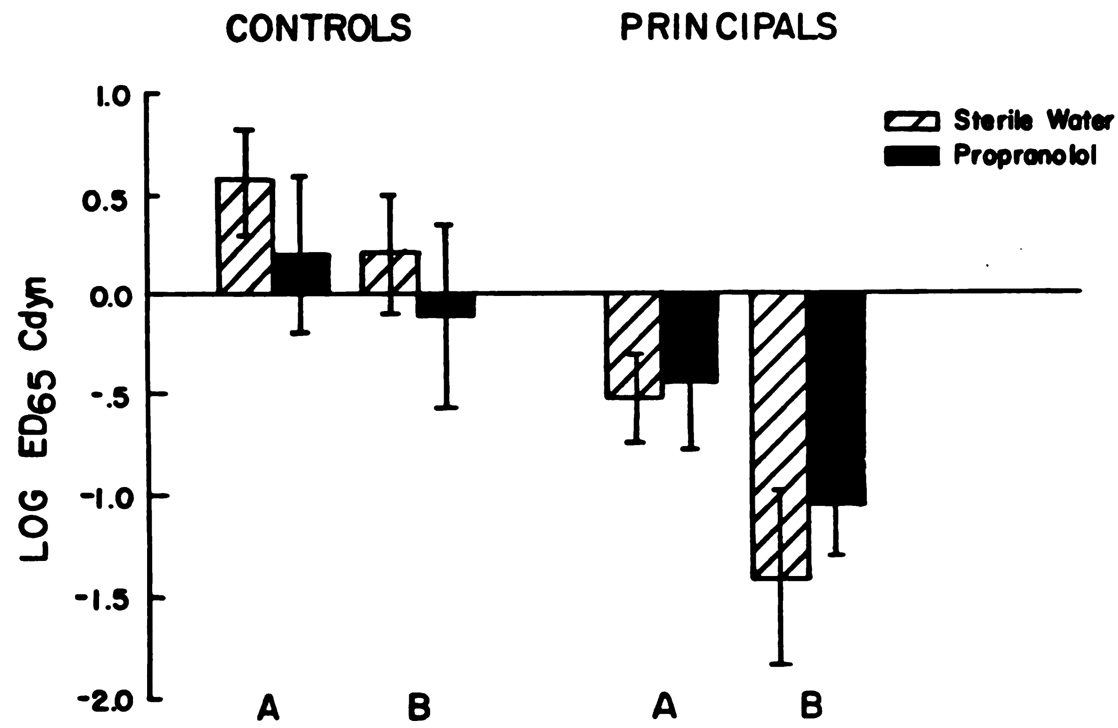

Figure 3-7 Aerosol histamine dose required to reduce dynamic compliance to 65% of baseline ($ED_{65}C_{dyn}$) following treatment with iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) in 6 principal and 6 control ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

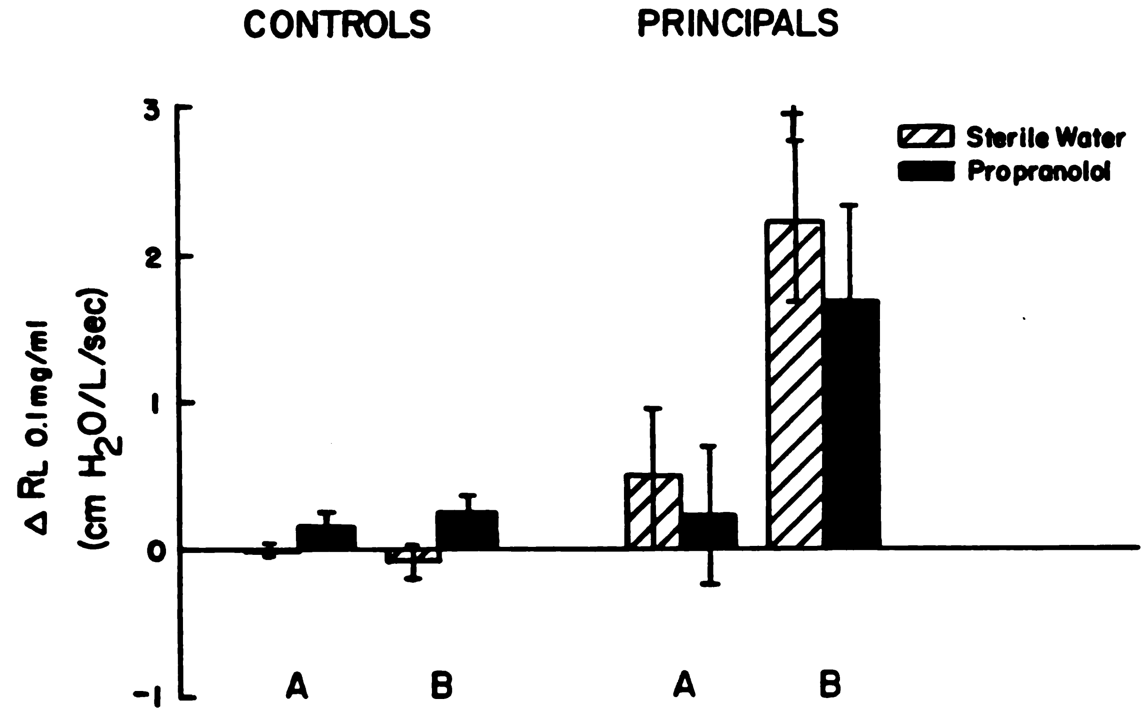

Figure 3-8 The change in pulmonary resistance between aerosol saline and 0.1 mg/ml aerosol histamine (delta $R_{L0.1}$ mg/ml) following administration of either iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) to 6 principal and 6 control ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction. +Significant difference between principal and control groups.

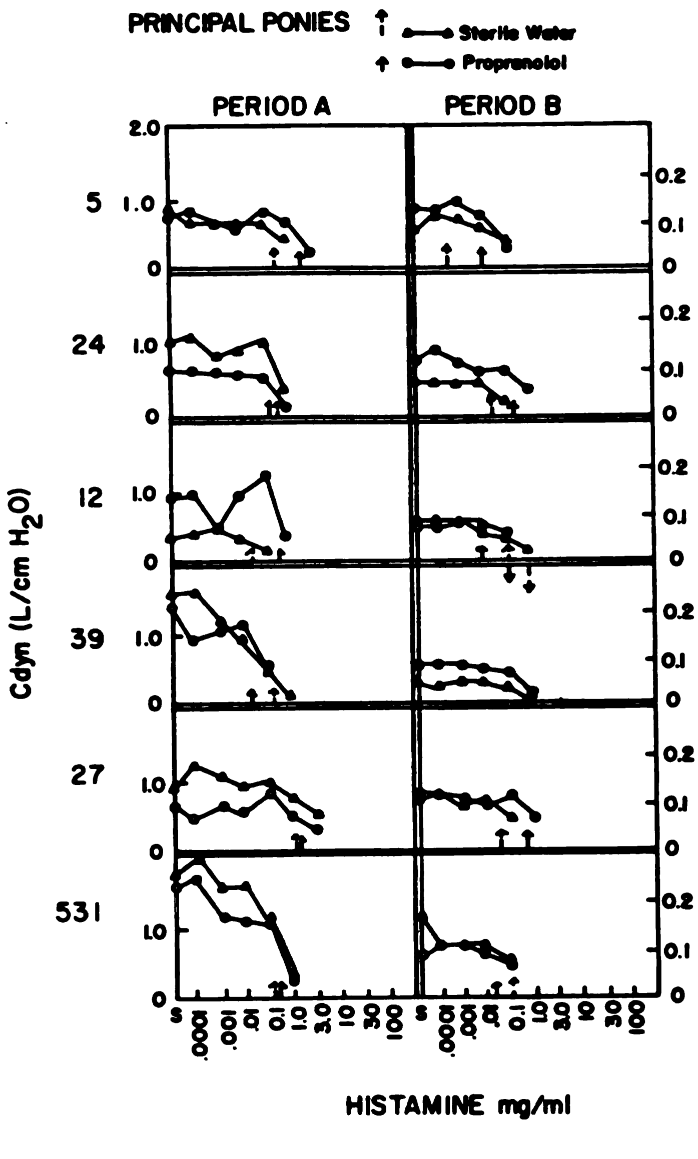

Figure 3-9 Dose response curves showing the effect of aerosol histamine on dynamic compliance (C_{dyn}) after iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) treatment in 6 principal ponies at time periods A and B. Arrows indicate the dose of histamine resulting in a decrease in dynamic compliance to 65% of baseline values ($ED_{65}C_{dyn}$). S, aerosol saline; period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

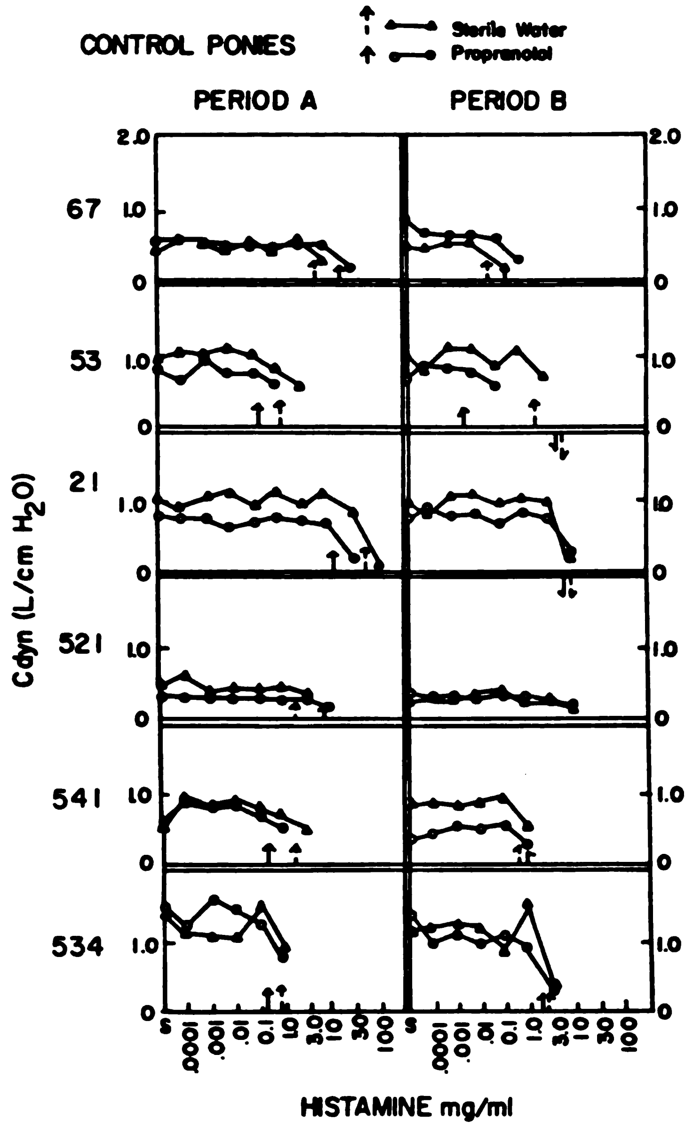

Figure 3-10 Dose response curves showing the effect of aerosol histamine on dynamic compliance (C_{dyn}) after iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) treatment in 6 control ponies at time periods A and B. Arrows indicate the dose of histamine resulting in a decrease in dynamic compliance to 65% of baseline values ($ED_{65}C_{dyn}$). S, aerosol saline; period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

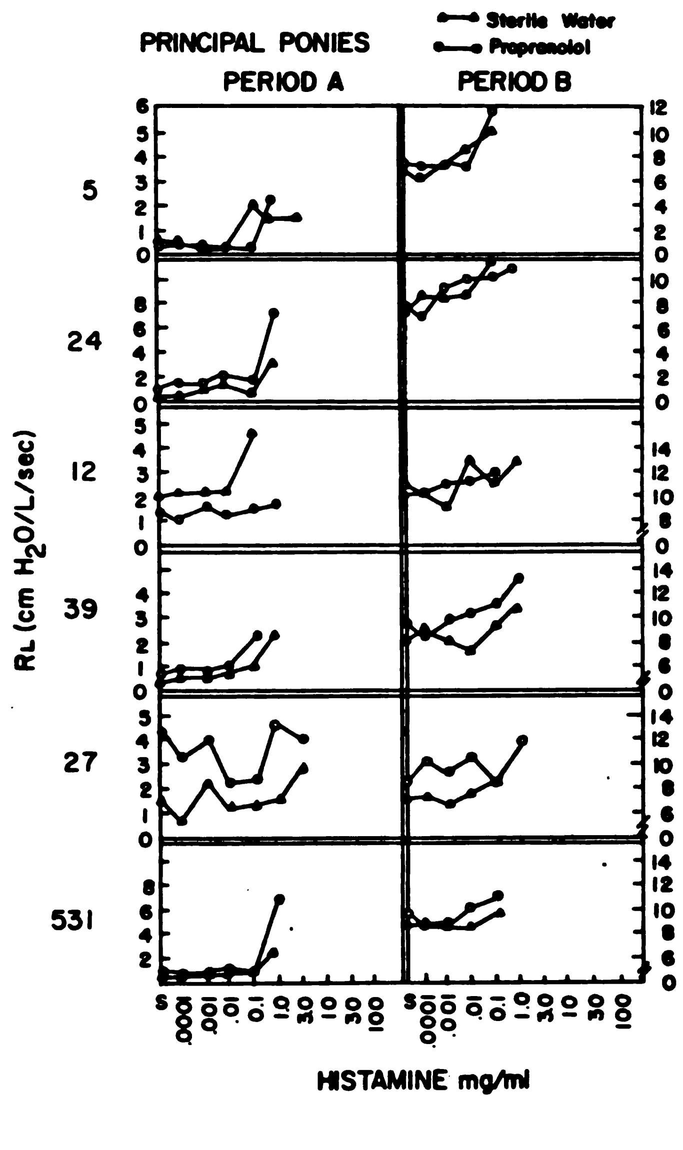

Figure 3-11 Dose response curves showing the effect of aerosol histamine on pulmonary resistance (R_L) after iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) treatment in 6 principal ponies at time periods A and B. S, aerosol saline; period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

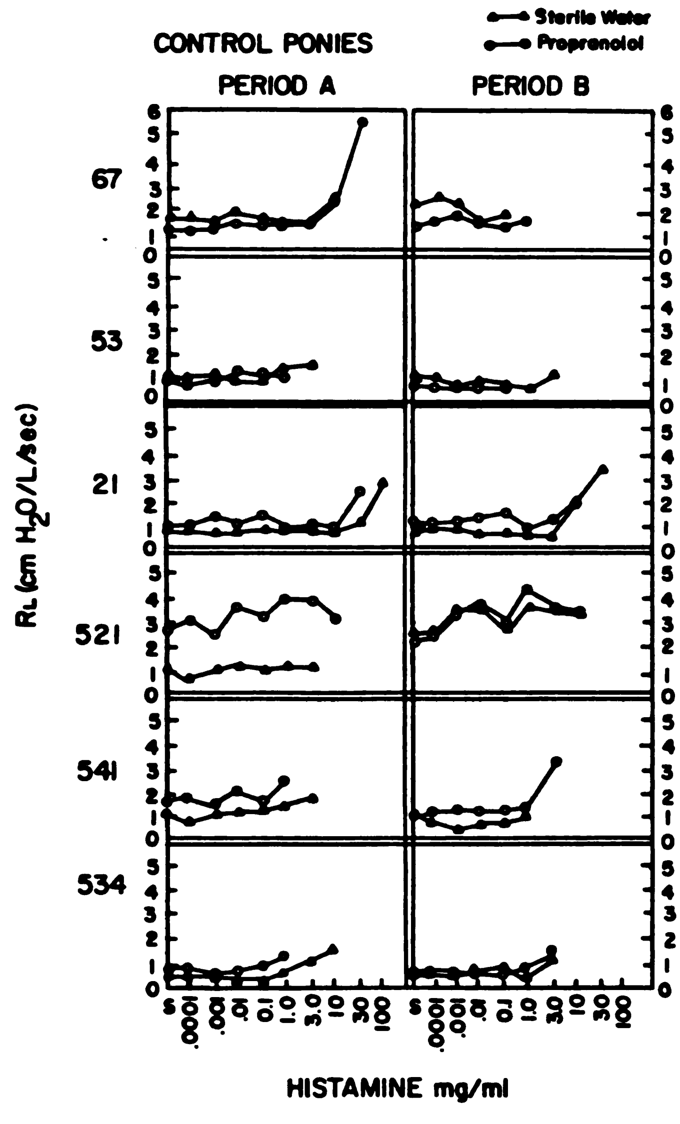

Figure 3-12 Dose response curves showing the effect of aerosol histamine on pulmonary resistance (R_L) after iv sterile water (20 ml) or iv propranolol (2.5 mg/kg) treatment in 6 control ponies at time periods A and B. S, aerosol saline; period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction.

Beta adrenergic blockade with propranolol did not change airway responsiveness to histamine in either group of ponies at period A or B. There was no significant change in either log $ED_{65}C_{dyn}$ (Figure 3-7) or delta $R_L0.1$ (Figure 3-8) following propranolol treatment.

Discussion

The results of this study show that when principal ponies are housed in a barn (period B) they develop airway obstruction manifested by decreased Cdyn and PaO_2 and increased R_L. During this acute disease exacerbation the principals have a further increase in R_L following propranolol administration. Studies with asthmatics similarly have demonstrated a decreased forced expiratory volume (FEV) following beta blockade (Grieco and Pierson 1971). The control ponies, however, do not exhibit bronchoconstriction at period B and like normal people were unaffected by beta adrenergic blockade with propranolol (Richardson and Sterling 1969). Beta adrenergic blockade did not change either group of ponies' responsiveness to administration of aerosol histamine.

The change in airway caliber induced by propranolol in the principal ponies was reflected only as an increase in pulmonary resistance without an accompanying decrease in dynamic compliance. The increase in R_L was consistent in every pony and did not occur during clinical remission or in the control animals. The increase in R_L with no change in C_{dyn} in the principal group, following beta blockade, suggests that during airway obstruction these ponies have an increased adrenergic drive predominantly to the central airways. The lack of an effect of propranolol on resting bronchomotor tone during clinical remission in the principal ponies and in the control ponies at both time periods indicates an absence of tonic adrenergic bronchodilator activity.

A defect in beta adrenergic receptor function has been proposed as a cause of the airway obstruction and bronchial hyperresponsiveness seen in asthmatics, and studies with ovalbumin-sensitized guinea pigs demonstrated a relative increase in alpha receptor numbers and lack of beta receptors in lung tissues (Barnes et al. 1980, Szentivanyi 1968). Differences between principal and control groups of ponies in the activity of beta adrenergic receptors occurred only at period B when principal ponies had airway obstruction. Whether a change in beta receptor numbers and/or activity is the cause of the different effect of propranolol on our two pony groups, cannot be determined by this study.

Studies in a guinea pig animal model of asthma have suggested that the mechanism of bronchoconstriction after propranolol administration is not due to its beta blocking effects but is due to the nonspecific side effects of the drug (Maclagan and Ney 1979, Ney 1983). However, other beta blocking drugs such as pindolol, which do not have for example, the local anesthetic effect of propranolol, also cause bronchoconstriction in asthmatics (Ruffin et al. 1979). Beta agonists also reduce release of mediators from mast cells, therefore it is possible that the mechanism of action of propranolol is through increased release of inflammatory mediators from mast cells (Peters et al. 1982).

Another factor involved in the airway obstruction seen in the principal ponies at period B could be an increased level of circulating catecholamines. Although data are not available for heavey ponies, asthmatics whether stable or during an acute asthmatic attack do not have an increase in circulating catecholamines (Ind et al. 1985).

Propranolol induced bronchoconstriction in asthmatics may result from unopposed intrinsic parasympathetic activity (Ind et al. 1989, Grieco and Pierson 1971). Ind and coworkers, demonstrated that an anticholinergic drug reversed and prevented propranolol induced bronchoconstriction in asthmatics (Ind et al. 1989). The authors concluded that the mechanism of action of beta adrenergic blockade may be through modulation of cholinergic transmission (Ind et al. 1989). Consistent with this theory, treatment of principal ponies with the anticholinergic agent, atropine, prevents the propranolol induced bronchoconstriction during the acute disease state (See Chapter 2). This indicates that the increase in R_L is most probably due to bronchoconstriction and may also be due to unopposed cholinergic activity. Beta adrenergic agonists in the dog have been shown to modulate cholinergic neurotransmission so that abolition of this modulation by propranolol could be the mechanism allowing the increase in parasympathetic tone (Vermiere and Vanhoutte 1979).

Sympathetic innervation of airways of many species is generally sparse (Doidge and Satchell 1982, Richardson 1979). Studies in guinea pigs have demonstrated sympathetic nerve fibers to tracheal smooth muscle but not to bronchial smooth muscle (Doidge and Satchell 1982, O'Donnel et al. 1978). Also, neurally mediated relaxation in this species is antagonized by beta blockers in tracheal but not bronchial smooth muscle. Furthermore, Broadstone and coworkers demonstrated an absence of sympathetic innervation to smooth muscle in vitro at the level of the third generation bronchi in heavey horses (Broadstone et al. 1989). Therefore, the effect of propranolol only on R_L may be due to the fact that horses have sympathetic innervation only to the smooth muscle of the larger central airways.

Propranolol administration in our control ponies did not alter the response to aerosol histamine challenge. These results are consistent with studies in mongrel dogs and nonasthmatic patients where beta blockade did not enhance bronchial hyperresponsiveness to aerosol challenge (Snapper et al. 1981, Zaid and Beall 1966). Although propranolol increased R_L in the principal ponies at period B, it had no effect on either measure of histamine responsiveness ($ED_{65}C_{dyn}$ or delta $R_{L0.1}$). This is similar to the lack of effect of beta blockade on the airway response to methacholine in Basenji-Greyhound dogs, another model of airway hyperresponsiveness (Hirshman et al. 1981). In the latter model, propranolol did, however, increase the bronchial response to citric acid, and beta blockade also increases responsiveness to methacholine in asthmatics (Zaid and Beall 1966). The lack of effect of propranolol in our principal ponies suggests that a partial beta blockade is not likely to be a significant mechanism in bronchial hyperresponsiveness to histamine.

In summary, the results of this study show that ponies with recurrent airway obstruction bronchoconstrict following beta blockade similar to asthmatics. The restriction of the effect of intravenous propranolol to R_L

suggests an increased adrenergic drive to the central airways. Since airway responsiveness to histamine did not change with propranolol, the beta adrenergic system does not seem to be involved in this phenomenon.

CHAPTER 4

Comparison of Aerosol Effects of Beta$_1$ And Beta$_2$ Adrenergic Antagonists in Ponies with Recurrent Obstructive Pulmonary Disease

Introduction

Lands et al. classified beta receptors in bronchial smooth muscle as the beta$_2$ type (Lands et al. 1967). However, since that time both beta$_1$ and beta$_2$ adrenergic receptors have been demonstrated in airways (Furchgott et al. 1975). Furthermore, the originally supposed cardioselective beta$_1$ adrenergic blockers have been shown to cause bronchoconstriction in some obstructive airways diseases (Johnsson et al. 1975, Bernecker and Roetscher 1970, Formgren 1972, Waal-Manning and Simpson 1971). I therefore wanted to determine if the effect of beta blockade with propranolol on ponies with recurrent obstructive pulmonary disease was due to either beta$_1$ and/or beta$_2$ adrenergic receptor mediated effects. Since beta adrenergic agonists have been shown to produce fewer systemic side effects and better bronchodilation when administered by inhalation than intravenously, I used aerosol administration of the beta$_{1\&2}$ adrenergic blocker propranolol, the beta$_1$ selective antagonist, atenolol, and the beta$_2$ selective antagonist, butoxamine, to clarify further the role of beta receptors in ponies with heaves (Thiringer and Svedmyr 1976, Popa 1984).

Experimental Design

The ponies in all three sections of this study were studied tranquilized with xylazine, standing in stocks. In the first section of the study, aerosol vehicle (20 ml) or aerosol propranolol at a dosage rate of 2.5 mg/kg was administered to 5 principal ponies at time periods A and B, and to 5 control ponies. Lung function measurements were performed at baseline and following administration of the vehicle or propranolol (post-blockade). Adequacy of the beta adrenergic blockade was confirmed by a lack of an increase in heart rate following the administration of aerosol isoproterenol (3 mg/ml). This was the dose of aerosol isoproterenol determined in pilot studies to be adequate to produce bronchodilation and an increase in heart rate with minimal systemic side effects such as pony excitability.

In the second section of the study I used six principal ponies only during acute disease exacerbations (period B) and five control ponies. Pulmonary function measurements were made before (baseline) and after aerosol administration of the specific $beta_1$ antagonist atenolol (post-blockade), at a dosage rate of 1.7 mg/kg. Adequacy of the $beta_1$ blockade was again confirmed by a lack of an increase in ponies' heart rate following 3 mg/ml of aerosol isoproterenol. Aerosol isoproterenol, prior to atenolol, increased the ponies' heart rate from an average rate of 60 beats/minute to 149 bpm. Administration of aerosol atenolol attenuated the increase in ponies' heart rate following isoproterenol administration (Figure 4-1).

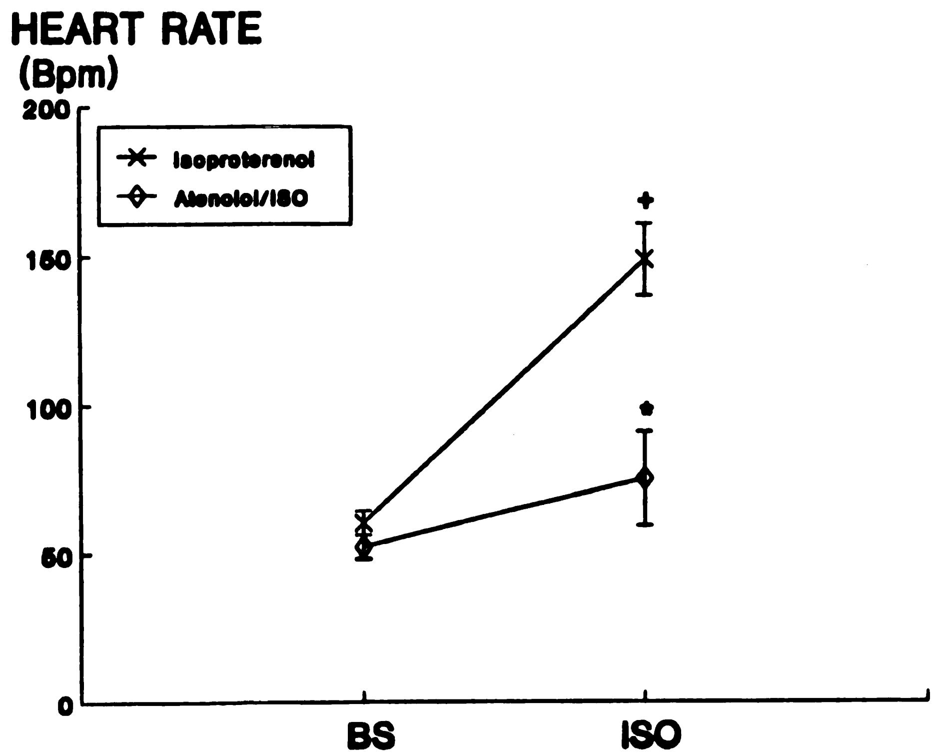

Figure 4-1 Heart rate in beats per minute (bpm) at baseline (BS) and after treatment with aerosol isoproterenol (3 mg/ml) (ISO) alone or aerosol isoproterenol (3 mg/ml) following blockade with atenolol in 5 control ponies. * Significant difference from isoproterenol alone. + Significant difference from baseline.

In the final section of this study, two heavey ponies at period B received the $beta_2$ adrenergic antagonist butoxamine via aerosol administration. Lung function measurements were made at baseline and following administration of aerosol butoxamine (post-blockade) at a dosage of 2.0 mg/ml. Adequacy of the $beta_2$ blockade was confirmed by a lack of bronchodilation following administration of aerosol isoproterenol (3 mg/ml).

A pilot study using two principal ponies at period B was also performed to determine the effect of atropine (0.02 mg/kg) on pulmonary function measurements following administration of the aerosol beta antagonists butoxamine and atenolol.

Pulmonary function measurements and aerosol delivery were performed as described in the material and methods section (See Chapter 1).

Statistical Analysis

Statistical analysis was performed using a single factor repeated measure and split plot analysis of variance (Steel and Torrie 1960). When F values were significant at $p < 0.05$, means were compared using Tukey's procedure (Steel and Torrie 1960).

Results

The principal ponies during acute obstructive disease (period B) were hypoxemic (Table 4-1) and had a significant decrease in C_{dyn} and increase in R_L when compared to remission (period A) values and the control group (Figures 4-2, 4-3, 4-4, and 4-5). There were no significant differences in VT, f, or $PaCO_2$ between the two groups of ponies, or between time periods A and B in the principal group, similar to the previous studies (Table 4-1).

Administration of aerosol propranolol did not change C_{dyn} (Figures 4-2 and 4-3) or R_L (Figures 4-4 and 4-5) in the control ponies or in the principal ponies at period A. In contrast, at period B, during an acute disease exacerbation, aerosol beta blockade with propranolol significantly increased R_L (2.91 $\pm$ .38 to 6.08 $\pm$ .87 cm H2O/L/sec) (Figure 4-5) and decreased C_{dyn} (.35 $\pm$ .05 to .08 $\pm$ .03 L/cm H2O) (Figure 4-3) in the principal ponies. These data differ from those in Chapter 3 in which beta adrenergic blockade with iv propranolol increased R_L but did not change C_{dyn} in the heavey ponies at period B.

TABLE 4-1 Baseline pulmonary function variables prior to vehicle or aerosol propranolol treatment in principal ponies at periods A and B and control ponies. Values are baseline means ± SEM. * Significant difference from period A. + Significant difference between principal and control groups.

	Measurement Period	
	A	B
	Principal Ponies	
PaO_2, torr	89.3 ± 1.7	69.2 ± 2.0*+
$PaCO_2$, torr	41.3 ± 0.8	40.8 ± 1.3
Tidal volume, liters	2.23 ± 0.1	1.87 ± 0.1
Frequency, min^{-1}	20.5 ± 2.6	30.6 ± 2.8
	Control Ponies	
PaO_2, torr	93.0 ± 3.2	
$PaCO_2$, torr	39.0 ± 1.8	
Tidal volume, liters	2.00 ± 0.1	
Frequency, min^{-1}	20.0 ± 2.0	

The change in C_{dyn} (delta C_{dyn}) from baseline values to post-blockade values following aerosol propranolol was -0.27 ± .04 while delta C_{dyn} following iv propranolol was -0.01 ± .01 (Figure 4-6). Delta C_{dyn} following aerosol beta blockade was significantly greater than delta C_{dyn} following iv beta blockade, suggesting an effect of aerosol propranolol on the peripheral airways in the heavey ponies during airway obstruction. Figure 4-7 shows the change in R_L (delta R_L) from baseline values to after treatment with either aerosol or iv propranolol in the principal ponies at period B. Delta R_L values recorded following aerosol and iv propranolol administration were not significantly different which suggests that the effect of beta blockade on the central airways was not dependent on the route of administration of propranolol.

In the second section of the study aerosol atenolol (beta$_1$ antagonist) was administered to control ponies, and to the principal ponies only at time period B. The principal ponies when housed in a barn were hypoxemic (PaO_2, 74.8 ± 1.95 mm Hg) and had a low C_{dyn} (.23 ± .05 L/cm H2O) and a high R_L (3.44 ± .35 cm H2O/L/sec) similar to values recorded in the first section of this study and in previous studies.

Aerosol administration of atenolol did not change C_{dyn} or R_L in the control ponies (Figure 4-8). The effect of beta$_1$ adrenergic blockade on C_{dyn} and R_L in the principal ponies at period B is shown in Figure 4-9. Atenolol administration significantly decreased C_{dyn} (.23 ± .02 to .15 ± .03 L/cm H2O) and increased R_L (3.44 ± .35 to 5.04 ± .62 cm H2O/L/sec) from baseline values.

In the final section of this study aerosol butoxamine ($beta_2$ antagonist) was administered to 2 principal ponies at period B. The principal ponies when housed in the barn had a low PaO_2 and C_{dyn} and a high R_L as seen in the previous section. $Beta_2$ adrenergic blockade increased R_L and decreased C_{dyn} (Figure 4-10) which suggests that $beta_2$ receptors are also partially mediating the increased R_L and decreased C_{dyn} that occurs following aerosol propranolol administration.

The change in C_{dyn} from values recorded at baseline to values recorded following administration of aerosol atenolol, aerosol butoxamine, and aerosol propranolol in the principal ponies at period B is shown in figure 4-11 . Delta C_{dyn} in response to aerosol propranolol was greater than delta C_{dyn} in response to either the aerosol atenolol $beta_1$ blockade or the aerosol butoxamine $beta_2$ blockade alone. However, the change in R_L from values recorded at baseline to values recorded following administration of aerosol atenolol was less than delta R_L to either aerosol propranolol or aerosol butoxamine (Figure 4-12). Delta R_L in response to aerosol propranolol and aerosol butoxamine, however, did not appear to be different.

Figure 4-13 demonstrates the effect of cholinergic blockade with atropine on C_{dyn} and R_L following the administration of aerosol atenolol or aerosol butoxamine in two ponies at period B. Atropine reversed the effect of atenolol on C_{dyn} and R_L but was without effect on the decrease in C_{dyn} and increase in R_L seen following butoxamine administration.

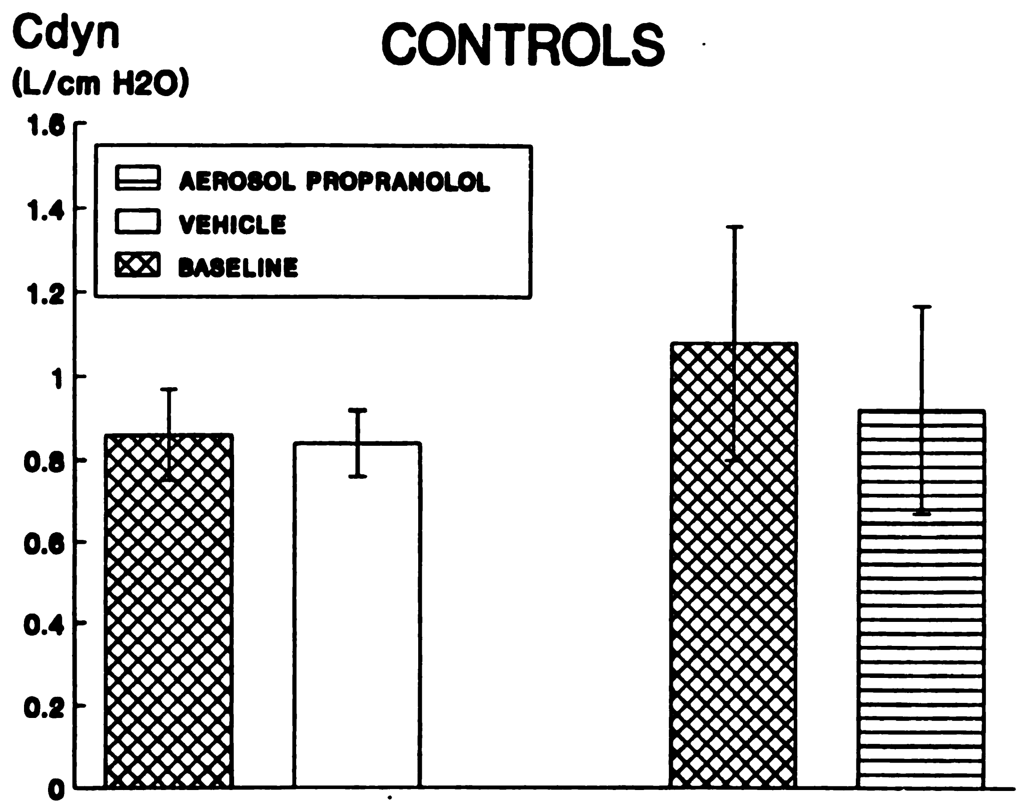

Figure 4-2 Dynamic compliance (C_{dyn}) at baseline and after treatment with aerosol vehicle (20 ml) or aerosol propranolol (2.5 mg/kg) in 5 control ponies.

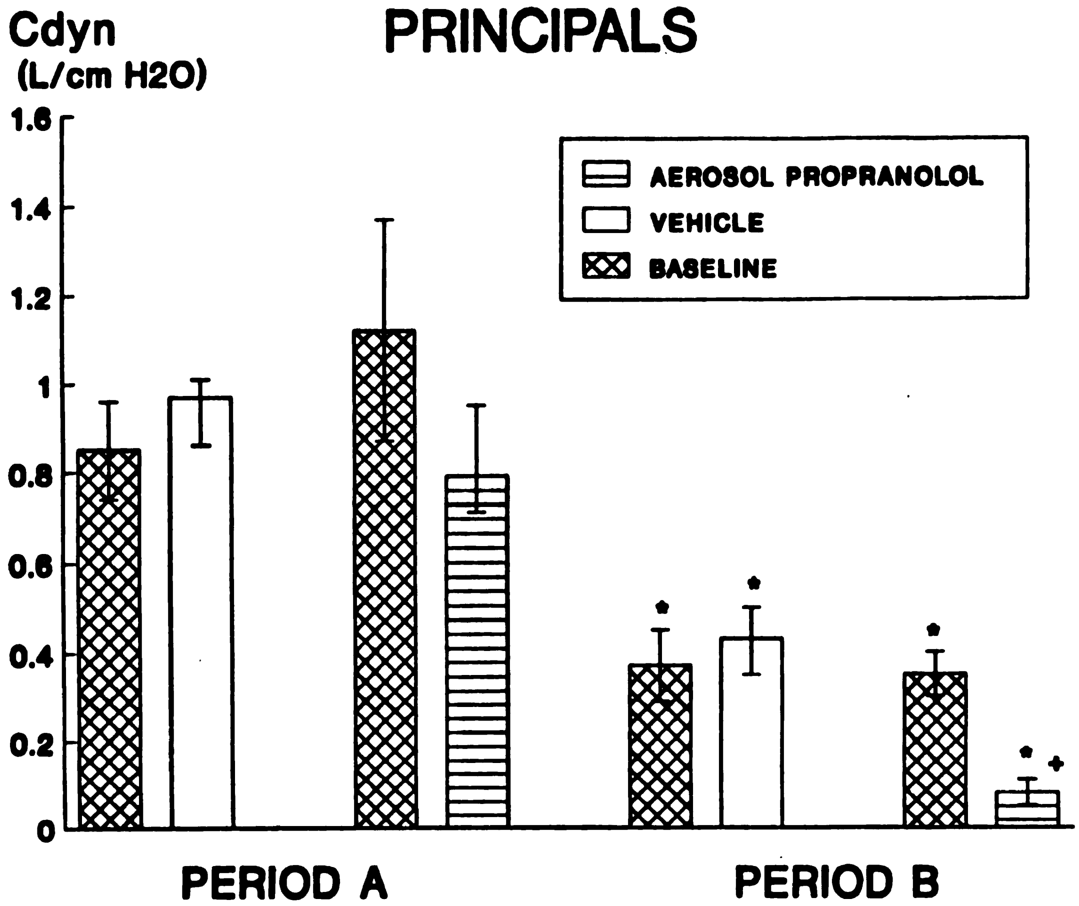

Figure 4-3 Dynamic compliance (C_{dyn}) at baseline and after treatment with aerosol vehicle (20 ml) or aerosol propranolol (2.5 mg/kg) in 5 principal ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction. * Significant difference from period A. + Significant difference between baseline and aerosol propranolol.

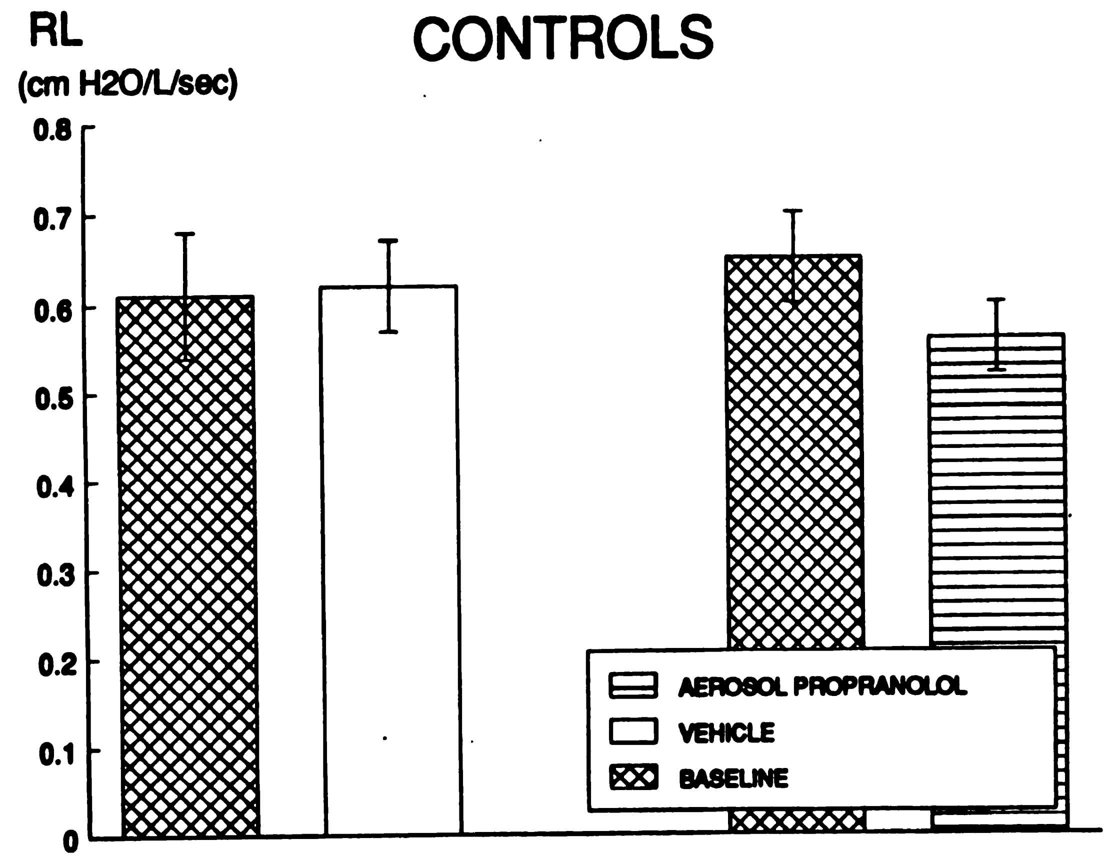

Figure 4-4 Pulmonary resistance (R_L) at baseline and after treatment with aerosol vehicle (20 ml) or aerosol propranolol (2.5 mg/kg) in 5 control ponies.

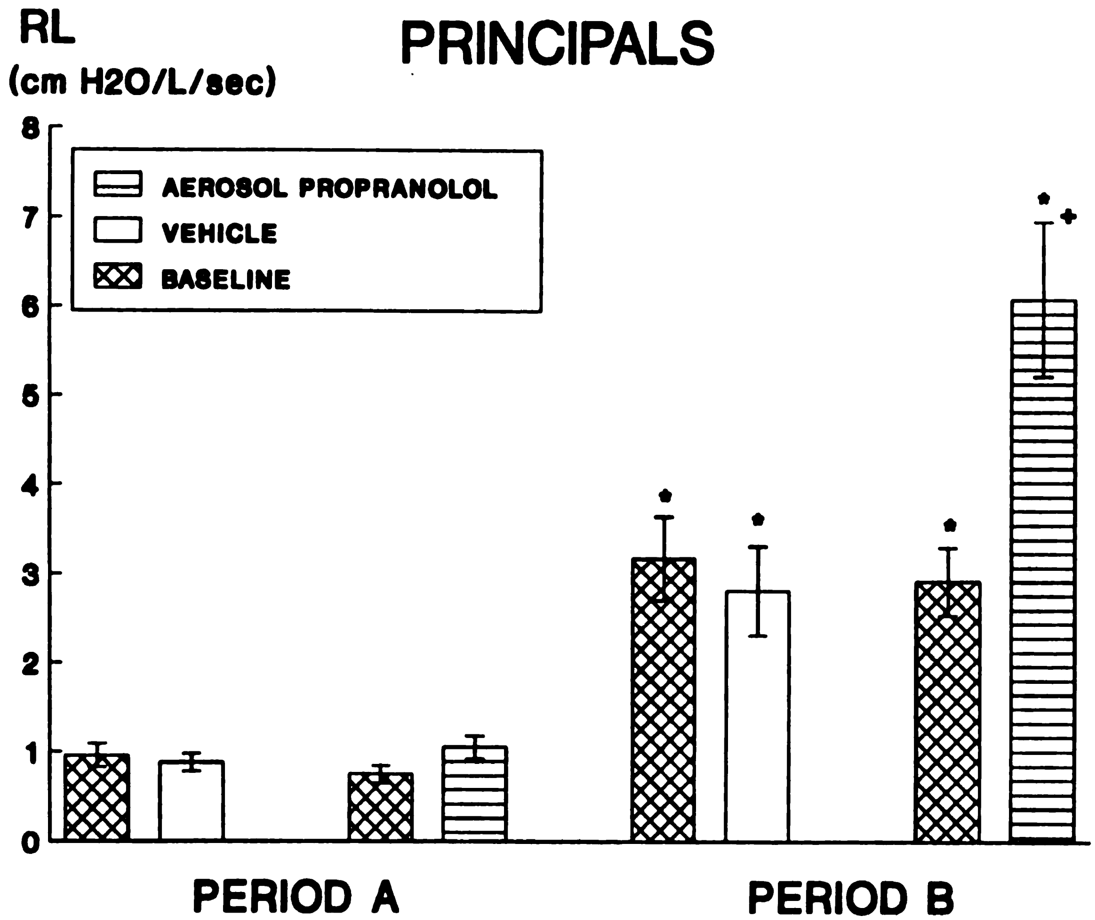

Figure 4-5 Pulmonary resistance (R_L) at baseline and after treatment with aerosol vehicle (20 ml) or aerosol propranolol (2.5 mg/kg) in 5 principal ponies at periods A and B. Period A, principal ponies in clinical remission; period B, principal ponies during acute airway obstruction. * Significant difference from period A. + Significant difference between baseline and aerosol propranolol.

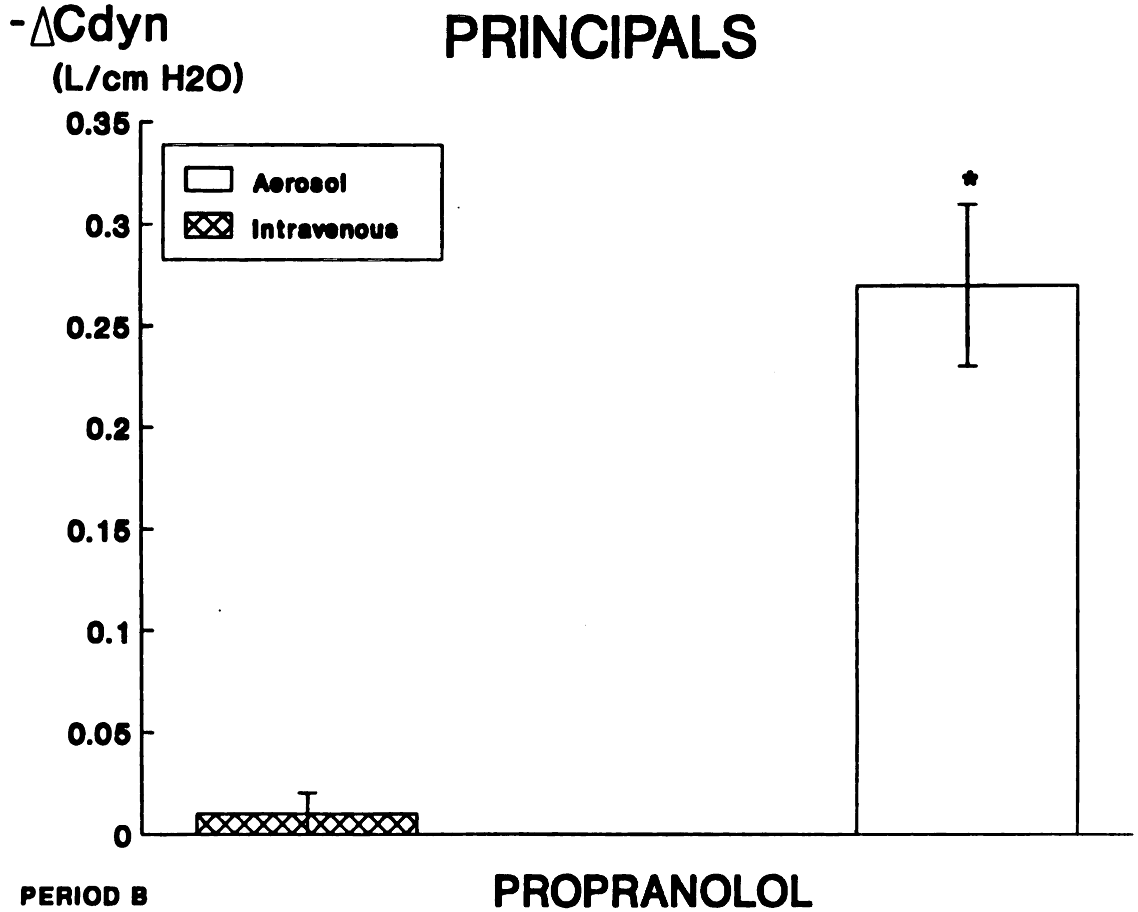

Figure 4-6 The change in dynamic compliance (-delta C_{dyn}) from baseline to after treatment with aerosol or iv propranolol (2.5 mg/kg) in principal ponies at period B (during airway obstruction). * Significant difference between iv and aerosol propranolol.

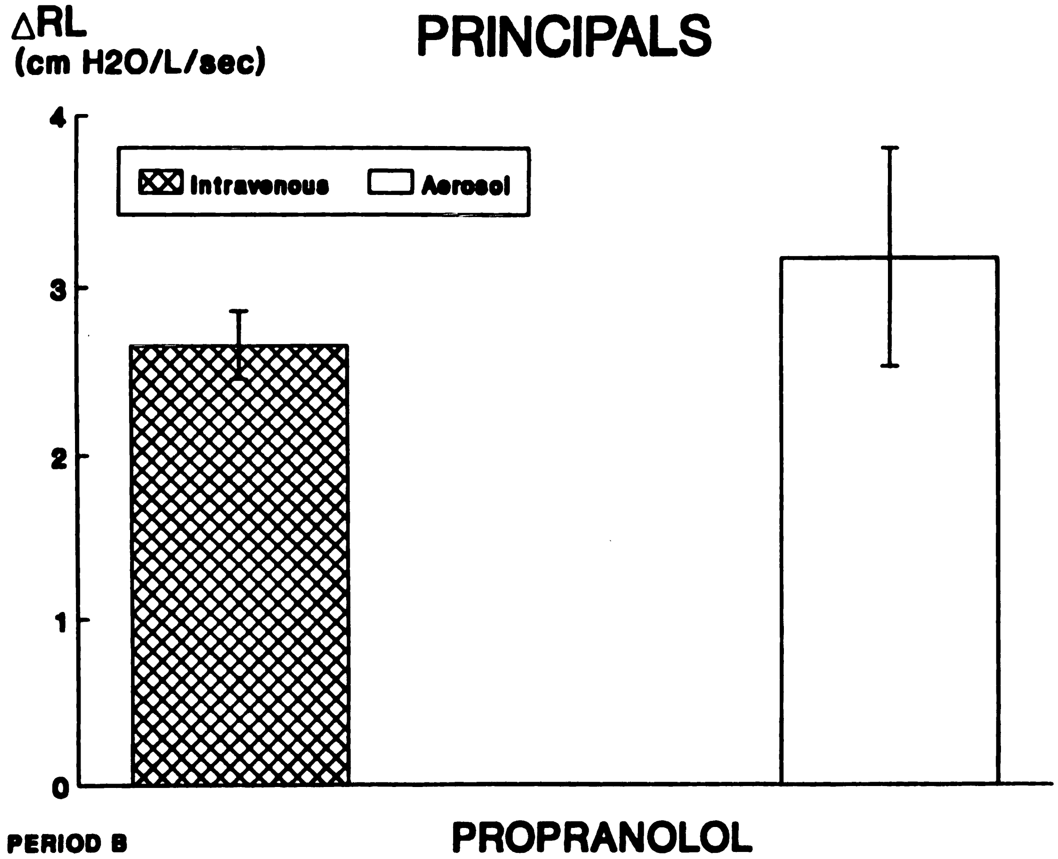

Figure 4-7 The change in pulmonary resistance (delta R_L) from baseline to after treatment with aerosol or iv propranolol (2.5 mg/kg) in principal ponies at period B (during airway obstruction).

CONTROLS

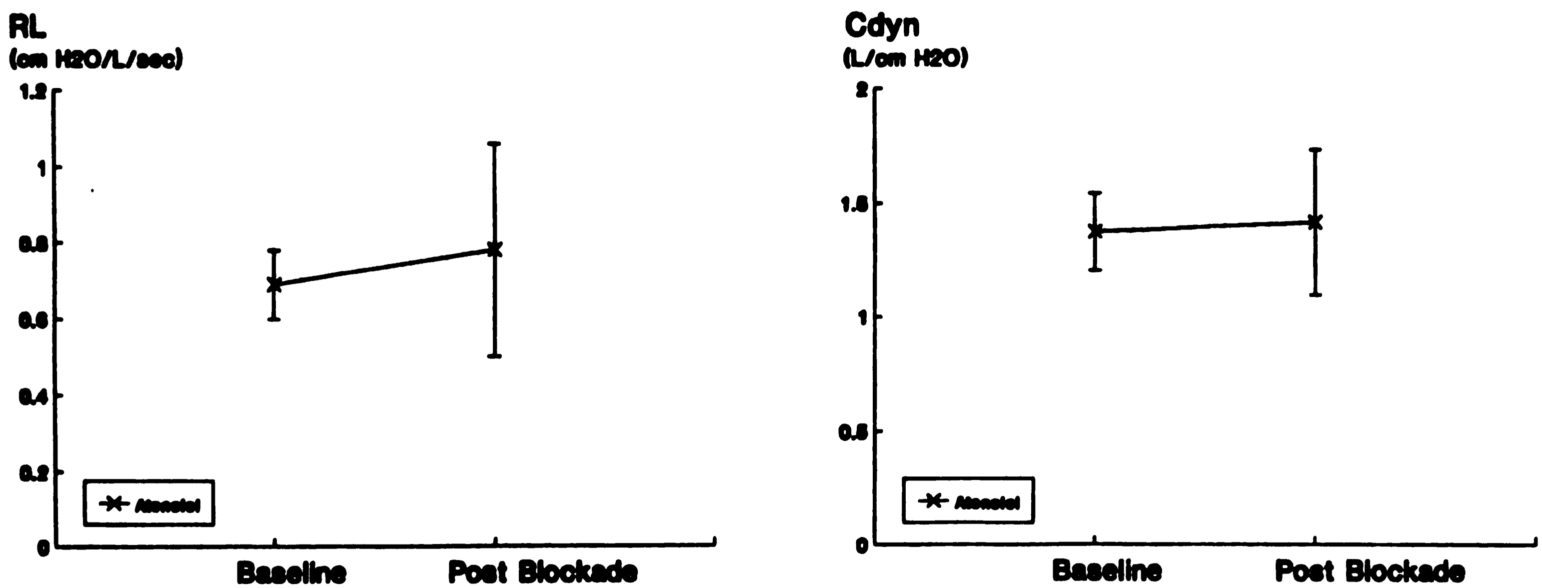

Figure 4-8 Dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) at baseline and after blockade with aerosol atenolol (1.7 mg/kg) in 5 control ponies.

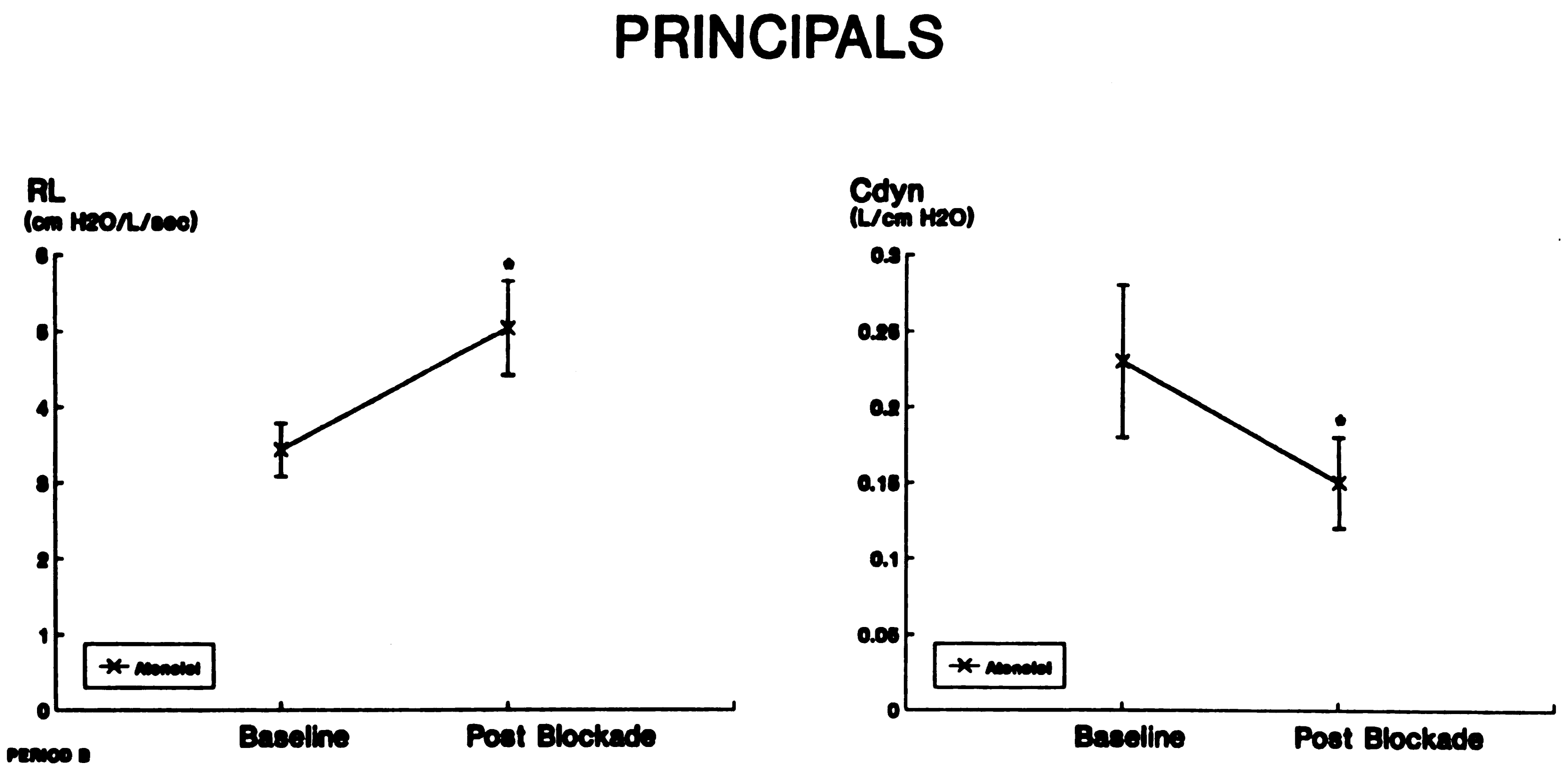

Figure 4-9 Dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) at baseline (BS) and after blockade with aerosol atenolol (1.7 mg/kg) in 6 principal ponies at period B (during airway obstruction). * Significant difference from baseline.

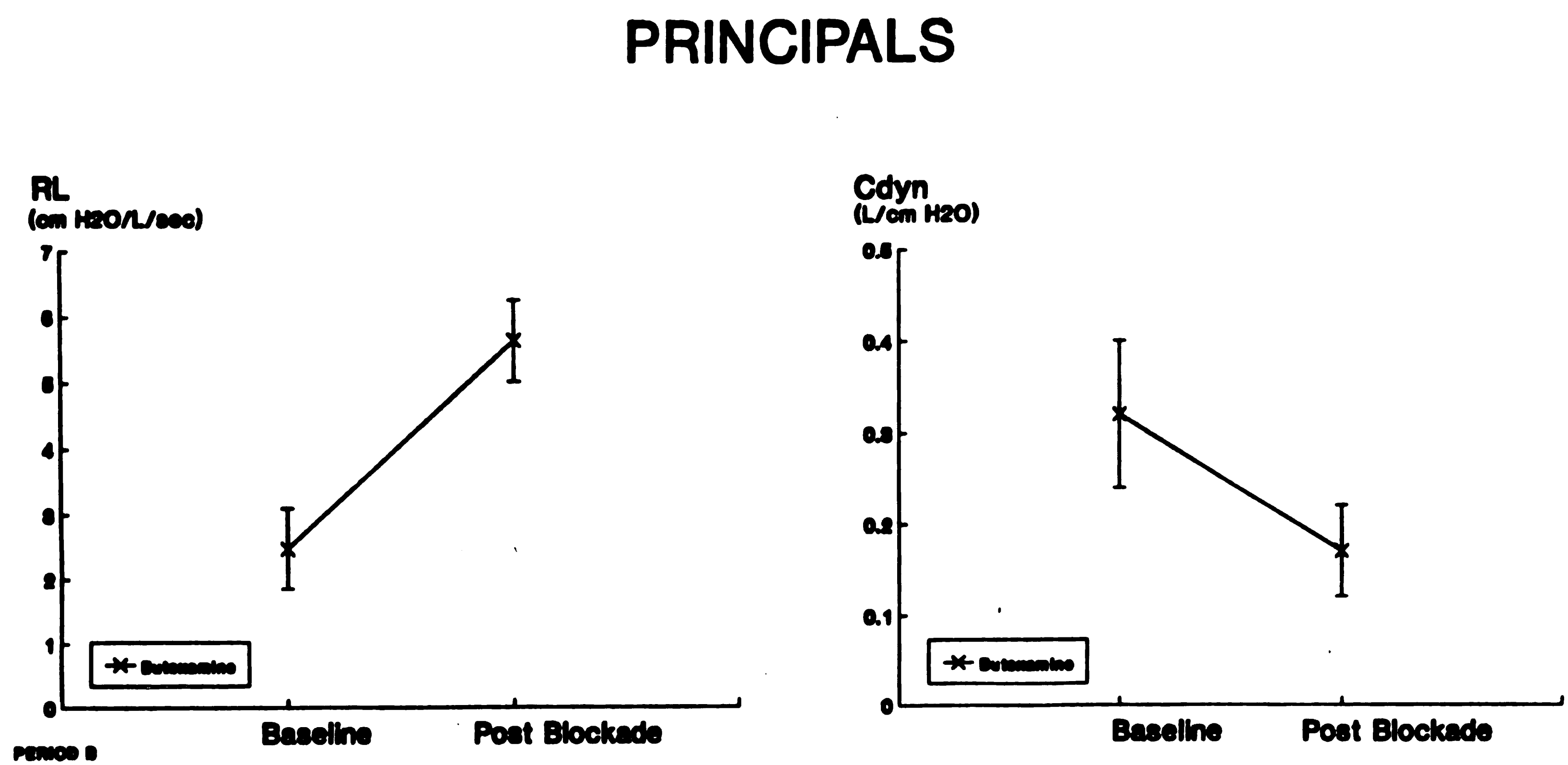

Figure 4-10 Dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) at baseline and after blockade with aerosol butoxamine (2 mg/kg) in 2 principal ponies at period B (during airway obstruction).

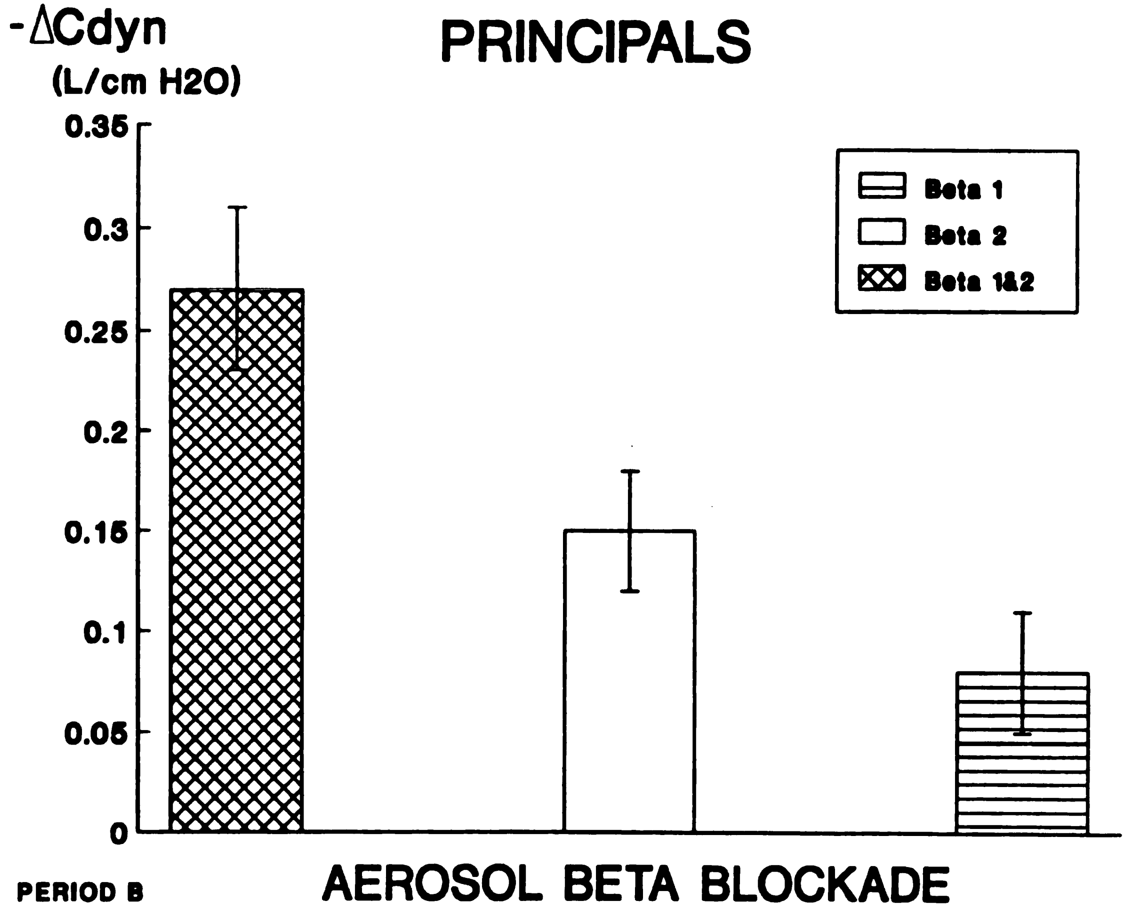

Figure 4-11 The change in dynamic compliance (-delta C_{dyn}) from baseline to after blockade with aerosol propranolol (beta$_1$ & beta$_2$) (2.5 mg/kg), aerosol butoxamine (beta$_2$) (2 mg/kg), aerosol atenolol (beta$_1$) (1.7 mg/kg) in principal ponies at period B (during airway obstruction).

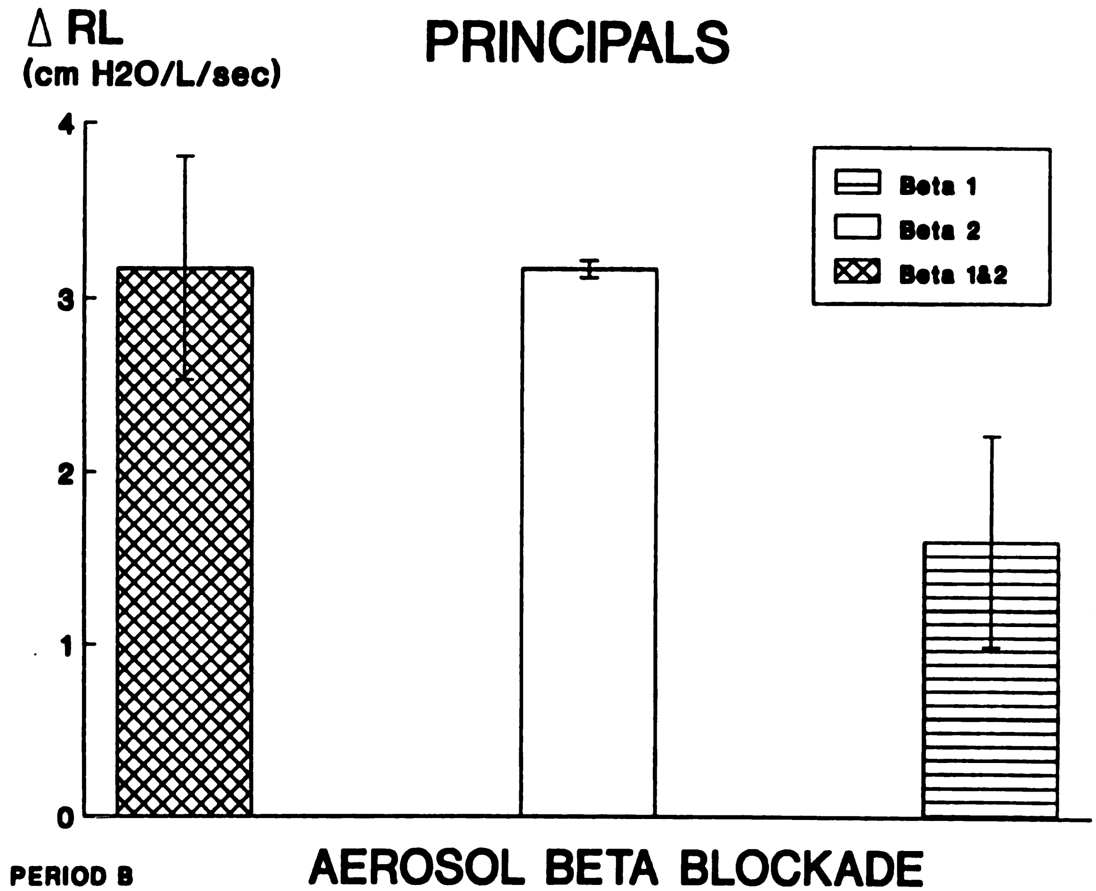

Figure 4-12 The change in pulmonary resistance (delta R_L) from baseline to after blockade with aerosol propranolol (beta$_1$ & beta$_2$) (2.5 mg/kg), aerosol butoxamine (beta$_2$) (2 mg/kg), aerosol atenolol (beta$_1$) (1.7 mg/kg) in principal ponies at period B (during airway obstruction).

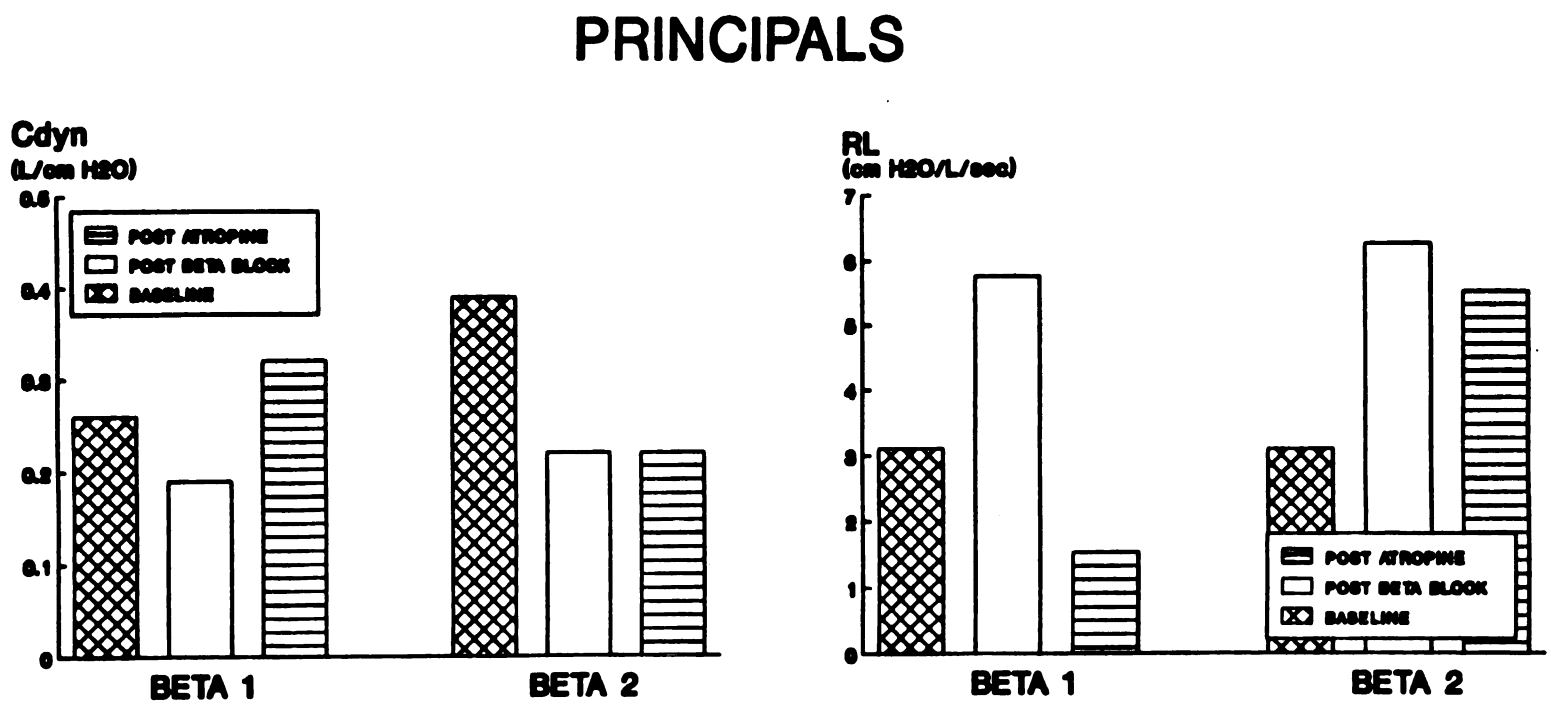

Figure 4-13 Dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) at baseline and after blockade with aerosol atenolol (1.7 mg/kg) ($beta_1$), aerosol butoxamine (2 mg/kg) ($beta_2$), and iv atropine (.02 mg/kg) plus aerosol atenolol (1.7 mg/kg) and aerosol butoxamine (2 mg/kg), in 2 principal ponies at period B (during airway obstruction).

Discussion

Consistent with previous studies, the principal or "heavey" ponies in this study when housed in a barn (period B) developed airway obstruction manifested by a decreased PaO_2 and C_{dyn} and an increased R_L. The control ponies did not exhibit airway obstruction when housed in a barn and were unaffected by beta adrenergic blockade with aerosol propranolol and atenolol. The control ponies are therefore similar to normal people who do not bronchoconstrict following beta adrenergic blockade (Richardson and Sterling 1969).

The change in airway caliber induced by aerosol propranolol in the principal ponies was reflected by both a decrease in C_{dyn} and an increase in R_L, whereas intravenous propranolol only increased R_L and did not decrease C_{dyn}. The effect of aerosol propranolol on C_{dyn}, the peripheral airways, and on R_L, the central airways, suggests beta adrenergic receptor activation not only to the central airways as seen in Chapter 3, but also to the peripheral airways.

The difference in effect on C_{dyn} of aerosol and iv propranolol could be due to the increased concentration of propranolol delivered to the airways by aerosol or possibly due to the action of the aerosol on the airway epithelium. It is possible that the concentration of propranolol delivered to the small airways is greater via aerosol than iv however, the airways of heavey ponies may be constricted and mucus plugged therefore decreasing the distribution of the aerosol.

With regard to the action of aerosols on airway epithelium, mechanical removal of the epithelium has been shown to increase the sensitivity of airway smooth muscle to various spasmogens (Vanhoutte 1988). Furthermore, the removal of epithelium decreases beta adrenergic agonist induced relaxation of airway smooth muscle in horses (Olson et al. 1989). The epithelium of airways produces a yet unknown epithelium derived relaxing factor (EpDRF) and has a high density of beta receptors that exceeds the density of receptors in the corresponding smooth muscle layers (Flavahan and Vanhoutte 1985, Carstairs et al. 1985, Agrawal et al. 1987, Xue et al. 1983). These data suggest that a facilitory role of the epithelial cells on beta adrenergic relaxation of airway smooth muscle could therefore be important for beta agonists administered by aerosol. This theory might also be true for the mechanism of action of beta adrenergic antagonists, therefore making propranolol more effective when administered by aerosol than intravenously. Adiministration of beta antagonists might therefore inhibit the release of EpDRF and/or mimic the effect of airway epithelium removal by blocking beta receptor responsiveness.

This study demonstrates that the increased R_L and decreased C_{dyn} which occurs following aerosol propranolol administration to principal ponies at period B is mediated by both $beta_1$ and $beta_2$ adrenergic receptors. The C_{dyn} results demonstrated both $beta_1$ blockade with atenolol and $beta_2$ blockade with butoxamine had less effect than the combined $beta_1$ and $beta_2$ blockade with propranolol in the peripheral airways (Figure 4-11). In contrast, the R_L results suggest that $beta_2$ blockade with butoxamine had a greater effect in the larger central airways than $beta_1$ blockade with atenolol, but the effect of butoxamine on R_L was not different from aerosol propranolol (Figure 4-12). This suggests a greater density and/or activity of $beta_2$ receptors than $beta_1$

receptors in the central airways but not in the peripheral airways of ponies with heaves.

As stated in Chapter 3 the effect of beta adrenergic blockade may be due to unopposed intrinsic parasympathetic activity, because atropine treatment prevents propranolol-induced airway obstruction (see Chapter 2, Figure 2-1). It is possible that the effect of atenolol could also be through modulation of cholinergic transmission since atropine reversed the airway obstruction that occurred following atenolol administration (Figure 4-13). Danser et al. using canine bronchi demonstrated that $beta_1$ adrenergic receptors inhibit cholinergic transmission (Danser et al. 1987). In contrast to Dansers' study, Rhoden and coworkers concluded that activated $beta_2$ adrenergic receptors can inhibit the release of acetylcholine on cholinergic nerves of human bronchi (Rhoden et al. 1988). Figure 4-13 however, shows that atropine administration did not reverse the $beta_2$ blockade induced airway obstruction in a principal pony at period B. These data suggest a minimal role of $beta_2$ adrenergic receptors in inhibiting cholinergic transmission in pony airways. The major portion of the increased R_L and decreased C_{dyn} following $beta_2$ blockade may therefore be due to a direct action of butoxamine on smooth muscle $beta_2$ receptors. This is supported by the fact that $beta_2$ adrenergic receptors mediate canine and human airway smooth muscle relaxation to exogenous beta agonists (Furchgott et al. 1975, Zaagsma et al. 1983). Relaxation of pony airway smooth muscle to beta agonists may also be mediated by $beta_2$ receptors.

In summary, the results of this study show that ponies with recurrent obstructive pulmonary disease bronchoconstrict following beta blockade with aerosol propranolol, atenolol ($beta_1$) and butoxamine ($beta_2$). The lack of an effect of aerosol beta blockade on pulmonary function in normal ponies confirms the results reported in Chapter 3. The effect of beta blockade on C_{dyn} and R_L suggests beta adrenergic activation in the central and also the peripheral airways of heavey ponies mediated through $beta_2$ and $beta_1$ adrenergic receptors. Furthermore, the mechanism of action for nonspecific beta blockade may be by modulation of cholinergic transmission via $beta_1$ receptors and also by direct action on smooth muscle receptors via $beta_2$ receptors.

CHAPTER 5

Beta$_2$ Adrenergic Attenuation of Histamine-Induced Airway Obstruction in Normal Ponies

Introduction

In canine and human airway smooth muscle, relaxation in response to exogenous beta agonists is mediated by beta$_2$ adrenergic receptors (Furchgott et al. 1975, Zaagsma et al. 1983). In contrast, in other species, such as the cat smooth muscle relaxation is mediated predominantly by beta$_1$ receptors (Silva and Ross 1974, O'Donnell and Wanstall 1983). The beta adrenergic receptor subtype(s) mediating smooth muscle relaxation of pony airways has never been determined in vivo. In vitro, beta adrenergic agonists, such as isoproterenol, have been shown to induce relaxation of normal and heavey horse tracheal smooth muscle precontracted with histamine (Olson et al. 1989, Robinson et al. 1989, Hanna and Eyre 1980). Furthermore, in an in vitro study of normal horse smooth muscle, relaxation of precontracted tissues was elicited by a nonspecific beta$_{1\&2}$ agonist and a beta$_2$ agonist to a similar extent, which leaves the beta receptor subtype function yet to be determined (Olson et al. 1989).

The previous study (Chapter 4) demonstrated that activation of beta$_1$ and beta$_2$ adrenergic receptors occurs in heavey ponies during an acute disease exacerbation however it did not determine which of these beta receptors mediates bronchodilation in ponies. To determine if beta$_1$ and/or beta$_2$ adrenergic receptors have a bronchodilator role in normal ponies in vivo, I tested the effect of beta agonists and $beta_1$ blockade on pony airways precontracted with iv histamine.

Experimental Design

Five mixed-breed normal ponies were studied tranquilized with xylazine, standing in stocks. The ponies' airways were constricted with iv histamine before receiving either aerosol isoproterenol ($beta_1$ & $beta_2$ agonist), aerosol clenbuterol ($beta_2$ agonist), or aerosol atenolol ($beta_1$ antagonist) plus isoproterenol. Aerosol atenolol was used prior to isoproterenol to determine if the bronchodilation seen with aerosol isoproterenol was a $beta_1$ and/or $beta_2$ mediated event. Four protocols were used in this study and the same ponies were used for each section. Each pony was tested on 4 separate days.

In the first protocol (histamine control), the ponies received an iv histamine continuous infusion at an average rate of 0.02 mg/kg/minute. The dose for a specific pony of iv histamine was considered adequate when C_{dyn} decreased to approximately 50% of the baseline value. To maintain bronchoconstriction the histamine infusion was continued throughout the protocols.

For the other three protocols, the ponies' airways were preconstricted with iv histamine before they received either aerosol clenbuterol, at a concentration of 36.4 micrograms/ml for 4 minutes, aerosol isoproterenol at a concentration of 3 mg/ml for 2 minutes, or aerosol atenolol (1.7 mg/kg) preceding aerosol isoproterenol.

Lung function measurements were taken at baseline, 15 minutes following the start of the iv histamine infusion (pretreatment constriction reading), and at 5 minute intervals following administration of the beta agonists. Following treatment with aerosol clenbuterol and in the histamine control, I repeated the pulmonary function readings at 5 minute intervals up to 25 minutes. However, following administration of aerosol isoproterenol, the lung function measurements were performed 5 and 10 minutes only. I was not able to continue these readings further due to the ponies' excitability. Aerosol isoproterenol increased the ponies' heart rate from a baseline of approximately 60 bpm to 149 bpm (See Chapter 4).

Lung function measurements and aerosol delivery were performed as previously described (See Chapter 1).

Statistical Analysis

Statistical analysis was performed using a split plot and single factor repeated measures analysis of variance. When F values were significant at $p < 0.05$, means were compared using Tukey's procedure (Steel and Torrie 1960).

Results

Histamine administered intravenously to normal ponies significantly decreased C_{dyn} (Figure 5-1) and increased R_L (Figure 5-2). In the absence of treatment with beta adrenergic agonists, airway constriction was maintained throughout the 25 minute duration of the protocol. The nonspecific beta agonist isoproterenol, and the $beta_2$ agonist, clenbuterol, both attenuated the iv histamine-induced decrease in C_{dyn} (Figure 5-1) and increase in R_L (Figure 5-2) to a similar extent. This demonstrates the involvement of beta adrenergic receptors in bronchodilation in pony airways. At the 10 minute pulmonary function reading, aerosol isoproterenol significantly increased C_{dyn} and decreased R_L when compared to the histamine control values and to the pretreatment constriction values. Aerosol clenbuterol significantly increased C_{dyn} and decreased R_L at the 10, 15, 20, and 25 minute function readings, when compared to the histamine control values. In addition, at 10 and 15 minutes following aerosol clenbuterol administration, C_{dyn} was significantly increased when compared to the pretreatment constriction values.

Administration of aerosol atenolol attenuated the increase in the ponies' heart rate seen following treatment with aerosol isoproterenol (See Chapter 4). While the $beta_1$ adrenergic blocker atenolol, blocked the $beta_1$ mediated increase in heart rate, it did not block the isoproterenol induced increase in C_{dyn} and decrease in R_L (Figure 5-3).

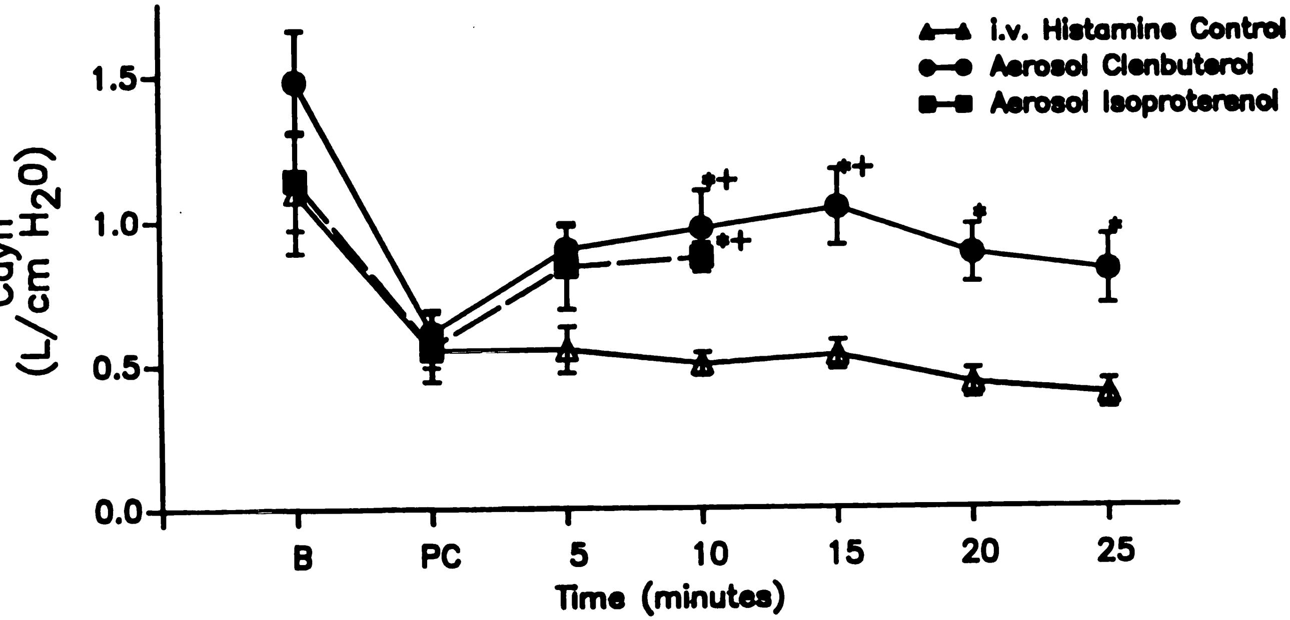

Figure 5-1 Dose response curves showing the effect of iv histamine (with and without beta agonist administration) on dynamic compliance (C_{dyn}) in 5 ponies. C_{dyn} was monitored for 25 minutes following administration of aerosol clenbuterol ($beta_2$) (36.4 micrograms/ml), 10 minutes following aerosol isoproterenol ($beta_1$ & $beta_2$) (3 mg/ml) and for 25 minutes for the iv histamine control (.02 mg/kg/min) B, baseline; PC, pretreatment values for constriction by iv histamine. * Significant difference from pretreatment constriction values. + Significant difference from histamine control values.

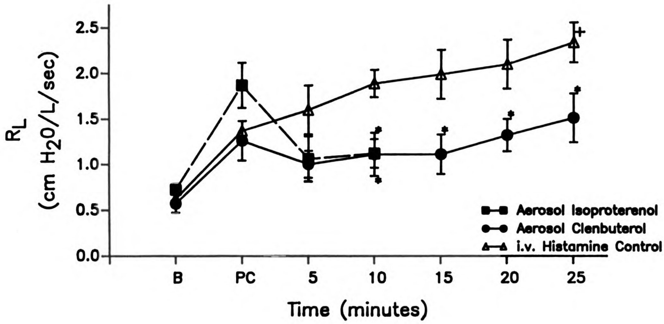

Figure 5-2 Dose response curves showing the effect of iv histamine (with and without beta agonist administration) on pulmonary resistance (R_L) in 5 ponies. R_L was monitored for 25 minutes following administration of aerosol clenbuterol ($beta_2$) (36.4 micrograms/ml), 10 minutes following aerosol isoproterenol ($beta_1$ & $beta_2$) (3 mg/ml) and for 25 minutes for the iv histamine control (.02 mg/kg/min) B, baseline; PC, pretreatment values for constriction by iv histamine. * Significant difference from histamine control values. + Significant difference from pretreatment constriction values.

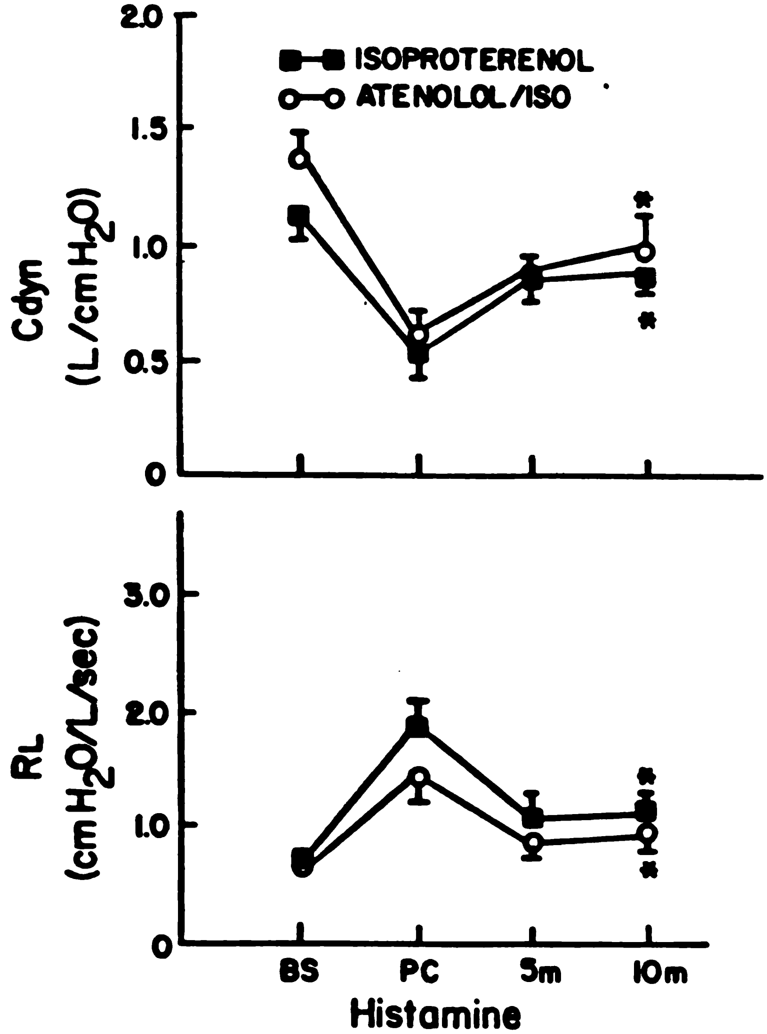

Figure 5-3 The effects of aerosol isoproterenol (3 mg/ml) alone, and isoproterenol plus $beta_1$ blockade with aerosol atenolol (1.7 mg/ml) on dynamic compliance (C_{dyn}) and pulmonary resistance (R_L) for 10 minutes following administration, in 5 pony airways preconstricted with iv histamine (.02 mg/kg/min). B, baseline; PC, pretreatment values for constriction by iv histamine. * Significant difference from pretreatment constriction values.

Discussion

The results of this study show that iv histamine administration to normal ponies in vivo results in airway obstruction that can be attenuated by a nonspecific $beta_{1\&2}$ agonist and a $beta_2$ adrenergic agonist to a similar extent. The $beta_1$ antagonist, atenolol did not block the bronchodilation seen with isoproterenol suggesting that the bronchodilation is largely a $beta_2$ mediated event.

Olson et al., also demonstrated that there was no difference between nonspecific ($beta_1$ & $beta_2$) agonist, and $beta_2$ agonist mediated in vitro relaxation of equine tracheal smooth muscle (Olson et al. 1989). However, the authors did not use a $beta_1$ antagonist in their experiments to rule out the possibility of $beta_1$ induced smooth muscle relaxation.

Murphy and coworkers demonstrated that administration of beta agonists to horses with heaves in vivo can cause bronchodilation, but the authors did not determine the beta adrenergic subtype (Murphy et al. 1980). In vitro, there are no differences in isoproterenol induced relaxation of normal and heavey horse airway smooth muscle (Robinson et al. 1989). However, Robinson et al. used a nonspecific beta agonist, isoproterenol, which did not determine the beta receptor subtype. In most species, beta agonist induced bronchodilation is mediated via $beta_2$ receptors (Barnes 1986a).

Studies using canine tracheal smooth muscle have shown that beta agonists cause relaxation mediated by $beta_2$ receptors only, and that sympathetic nerves relax airway smooth muscle via $beta_1$ receptors (Furchgott et al. 1975). This suggests that canine $beta_2$ receptors are regulated by circulating catecholamines, whereas $beta_1$ receptors are regulated by sympathetic nerves, however, the demarcation between the receptor types is probably not that clear. Furthermore, norepinephrine when released from sympathetic nerve endings can activate prejunctional $beta_1$ receptors to inhibit cholinergic transmission in canine bronchi (Danser et al. 1987). In ponies, beta agonists may also relax airway smooth muscle via $beta_2$ receptors only, as the results of this study suggest. Atenolol, the specific $beta_1$ antagonist, could not inhibit the bronchodilation following isoproterenol. Also, the degree of bronchodilation seen with clenbuterol, the $beta_2$ agonist, and isoproterenol was similar. This suggests a beta agonist induced activation primarily of $beta_2$ receptors.

In summary, aerosol clenbuterol and isoproterenol attenuated the iv histamine-induced airway obstruction in normal ponies. Furthermore, this attenuation of airway obstruction seems to be primarily a $beta_2$ adrenergic mediated event, since the $beta_1$ antagonist atenolol did not block the bronchodilation seen with isoproterenol.

CHAPTER 6

Summary and Conclusions

Abnormalities in the autonomic nervous system have been postulated as one possible mechanism of airway obstructive diseases (Szentivanyi 1968). I have investigated the $alpha_1$ and beta adrenergic systems in vivo in normal ponies, and ponies with "heaves" to try to determine if abnormalities of these systems might be part of the mechanism that lies between the unknown cause of heaves and the end result of bronchoconstriction and airway hyperresponsiveness. Conclusions and the discussion of each study will be summarized in this chapter.

When ponies with recurrent obstructive pulmonary disease are housed in a barn and fed moldy hay, they develop airway obstruction manifested by a decreased PaO_2 and C_{dyn}, increased R_L and hyperresponsive airways. The control ponies however do not develop airway obstruction when housed and fed in a manner similar to the principal ponies. These findings were consistent in each of my studies.

Using the $alpha_1$ receptor agonist, phenylephrine, I demonstrated a difference in $alpha_1$ receptor responsiveness between ponies with heaves and normal ponies whether the principal ponies were in remission or experiencing airway obstruction. These findings suggest either an increased density and/or activity of $alpha_1$ receptors in ponies with heaves. However, negative results were obtained when the $alpha_1$ receptor antagonist prazosin was administered to the principal ponies during an acute disease exacerbation to determine if the $alpha_1$ receptors were activated and contributing to the bronchoconstriction of heaves. Prazosin did not significantly increase C_{dyn} or decrease R_L which suggests that the role of $alpha_1$ receptors in the airway obstruction of heaves is minimal.

Beta adrenergic blockade with intravenous propranolol demonstrated that there is beta adrenergic receptor activation to the central airways of heavey ponies during airway obstruction but not in remission, or in control ponies. Propranolol did not however, have any effect on airway responsiveness to histamine which suggests that the beta adrenergic system is not involved in histamine hyperresponsiveness in ponies. The fact that the cholinergic antagonist, atropine, prevents propranolol induced bronchoconstriction in the heavey ponies, suggests that the airway obstruction caused by beta blockade may be the result of unopposed intrinsic parasympathetic activity. Therefore, one of the functions of beta adrenergic receptors in the airways of ponies may be presynaptic modulation of cholinergic transmission as is seen in other species (Danser et al. 1987, Rhoden et al. 1988).

Beta blockade with aerosol propranolol demonstrated that heavey ponies have beta receptor activation in both the peripheral and the central airways during an acute disease exacerbation only. Propranolol had no effect on the airways of the control ponies which suggests an absence of beta receptor activation but these data do not determine the presence or number of beta receptors. The fact that aerosol propranolol elicited a greater response in the airways (decreased C_{dyn}) than iv propranolol suggests that beta receptors on airway epithelium may play an important role in modulating airway caliber.

Beta$_1$ and beta$_2$ adrenergic receptors are both activated in ponies with recurrent obstructive pulmonary disease since the specific beta$_1$ and beta$_2$ blockers caused bronchoconstriction. Beta blockade again had no effect on normal ponies airways. Atropine administration reversed the bronchoconstriction seen following beta$_1$ blockade but not beta$_2$ blockade. This suggests that the role of beta$_1$ receptors in heaves may be presynaptic modulation cholinergic transmission while beta$_2$ receptors may function directly on the smooth muscle. Similarly, prejunctional beta$_1$ adrenergic receptors inhibit cholinergic transmission in canine bronchi and beta agonists relax canine airway smooth muscle via beta$_2$ receptors (Danser et al. 1987, Furchgott et al. 1975).

Aerosol beta adrenergic agonists administered to normal ponies attenuated iv histamine-induced airway obstruction and this attenuation was primarily beta$_2$ adrenergic receptor mediated. These data suggest that the primary beta receptor mediating smooth muscle relaxation in response to beta agonists is the beta$_2$ adrenergic receptor.

In conclusion, ponies with recurrent obstructive pulmonary disease have increased alpha$_1$ adrenergic receptor numbers and/or activity, but the role of alpha$_1$ receptors in heaves is minimal. Whether beta receptor numbers were changed in ponies with heaves could not be determined by this study. Ponies with recurrent obstructive pulmonary disease however do have beta adrenergic receptor activation during acute disease exacerbations. In addition both $beta_1$ and $beta_2$ receptors are activated during heaves. $Beta_1$ receptors may function to inhibit cholinergic transmission in the airways while $beta_2$ receptors might relax smooth muscle directly. Beta adrenergic receptors do not appear to have a role in airway responsiveness to histamine.

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