Evaluation of Guinea pig Models of the Acute Phase of Allergic Rhinitis

by

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B.Sc., Sultan Qaboos University, 2003

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Pharmacology & Therapeutics)

UNIVERSITY OF BRITISH COLUMBIA

April 2006

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Abstract

Allergic rhinitis is an allergen-IgE complex mediated inflammation of the nasal mucosa characterized by the symptoms of sneezing, nasal itchiness, rhinorrhea, and nasal congestion. The economical and social impact of allergic rhinitis is substantial. The effectiveness of currently available medications is limited. Investigation of more effective medications with fewer side effects is essential. Therefore, this study was intended to establish a model of allergic rhinitis in guinea pigs that can be utilized for further investigation of new medications. Furthermore, this study was also aimed to systematically evaluate the role of some inflammatory mediators of acute allergic reactions in guinea pigs in vivo.

Male Dunkin Hartley guinea pigs were intranasally sensitized to, and challenged with, ovalbumin. Sneezing (SN) and nose rubbing (NR) were evaluated on day 21 post initiation of sensitization dose. From day 23 after first sensitization, the animals were anaesthetized with intraperitoneal pentobarbital (30-35mg/kg). The trachea was cannulated in both directions, caudally for measurement of nasal airway pressure (NAP) using a ventilator flow method (8ml/beat, 72beats/min) and rostrally for measurement of lung inflation pressure (LIP). Drugs were administered prior to ovalbumin challenge. SN and NR were evaluated for 30 minutes and NAP was evaluated within 30 minutes post challenge. Cellular infiltration (CI) was assessed from nasal lavage collected 60 minutes post challenge.

Sensitized guinea pigs produced symptoms of SN, NR and nasal blockade (NB) in addition to eosinophil infiltration following ovalbumin challenge. A first generation H1 antihistamine, mepyramine, inhibited SN only, whereas later H1 antihistamine, cetirizine,
inhibited SN, NR and NB. Montelukast, a leukotriene D4 receptor antagonist, and heparin prevented NB and CI. L-NAME, a non specific nitric oxide synthase inhibitor, inhibited NB and stimulated neutrophil infiltration.

In non-sensitized guinea pigs, histamine and acetylcholine introduced intravenously caused dose-dependent decreases in NAP (by the action of histamine on H1, M2 and perhaps M5 receptors, and acetylcholine on M1 receptors) and increases in LIP (by the action of histamine on H1 receptors, and acetylcholine on M1 receptors).

In conclusion pathophysiological changes due to allergic rhinitis in guinea pigs resemble to some extent those in humans. The models reported here reflect the effectiveness of some drugs currently used to treat allergic rhinitis. The models can be used in investigating new potential drugs for the treatment of allergic rhinitis.
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Bismillah AL-Rahman AL-Rahim

In the name of God, the Beneficent, the Merciful
Acknowledgements

I would like to thank Professor M.J.A. Walker for his supervision, guidance, support and valuable directions throughout my study. I would like also to express my thanks to Ying Dong for her technical and experimental support. A great appreciation is given to my graduate committee members Dr. Catherine C.Y. Pang and Dr. Darryl A. Knight for their valuable ideas and suggestions. I also thank the undergraduate students Michelle Soh and David Ko for their help during experiments.

I owe my profound gratitude and special thanks to my wife who has been very patient and a source of emotional and moral support throughout the course of this research work.

My graduate research training would not have been possible without the award of the scholarship from Sultan Qaboos University, Oman. I would like to express my deepest thanks and appreciation to my country Oman, for providing me with this chance.

My experiments would not have been possible to conduct without the research fund from Rhinopharma Ltd. I owe the company thanks.
1 Introduction

This thesis describes the evaluation of models of rhinitis in guinea pigs in terms of the hallmarks of rhinitis, namely sneezing, itching, nasal secretions, nasal obstruction and leukocyte infiltration. The following introduction covers the relevant subjects including:

Rhinitis—its clinical and experimental characteristics;
Anatomy of the nose in humans and experimental animals;
The relevant nasal physiology in humans and experimental animals;
The pathophysiology of rhinitis—clinically and experimentally;
Current drug treatment for rhinitis—clinical and experimental;
Experimental models of rhinitis

The thesis will describe the various models that I have developed and used. Thereafter the results of the initial studies into the nature of the models will be discussed, followed by a description of the actions of drugs currently used in the clinic.

1.0 Allergic rhinitis

1.0.1 Human allergic rhinitis

Allergic rhinitis is defined as an abnormal inflammation of the membrane lining the nose. It is characterized by nasal congestion, rhinorrhea, sneezing, itching of the nose and/or postnasal drainage (Bousquet et al., 2001). Additionally, airway hypersensitivity (AHR) may develop, and loss of the sense of smell and an inability to taste may occur.
Moreover, some patients experience sleep disturbances, decreased emotional well-being and social functioning, headache, and irritability. On physical examination, nasal obstruction often can be seen, with pale to bluish nasal mucosa, enlarged or boggy turbinates, clear nasal secretions, and pharyngeal cobblestoning (streaks of lymphoid tissue). Other characteristic signs of allergic rhinitis in children include allergic shiners (darkening of the lower eyelids due to the edematous nasal tissue that compresses the veins that drain the eyes, leading to pooling of blood under the orbits) and the allergic crease (transverse skin line below the bridge of the nose) that is caused by constant rubbing upwards from the palm of the hand ("allergic salute"). Due to the chronic nasal airway obstruction, some children are chronic mouth breathers, which can lead to craniofacial abnormalities and orthodontic disturbances, such as palatal arching, increased facial length, and a flattened mid-face. Many patients do not show all these abnormalities, although they often may be sneezing and have rhinorrhea with mucosal edema (Nayak et al., 2001; Todd et al., 2005).

Although the onset of allergic rhinitis may occur at any age, it is most common in children and at adolescence. There is a decrease in incidence with advancing age. The financial impact of allergic rhinitis is significant. In 1996, the overall direct costs of treating allergic rhinitis exceeded $3 billion, with an additional $4 billion spent to treat related comorbidities triggered or exacerbated by the disease. Allergic rhinitis is the most common atopic disorder in the United States. It affects about 24 million (8% of the population) with an equal distribution between males and females. The prevalence of allergic rhinitis varies by age: 32% of patients are 17 years of age or younger, 43% are 18
to 44 years of age, 17% are 45 to 64, and only 8% are 65 years of age or older. The cost of treating allergic rhinitis plus indirect costs of the disorder, such as lowered productivity and time lost from work or school are substantial. In the United States alone, the number of lost workdays resulting from allergic rhinitis is estimated as approximately 3.5 million a year and the total direct health care cost of treating allergic rhinitis is estimated at $3.4 billion (Law et al., 2003; Holgate et al., 2003; Todd et al., 2005). Epidemiologically, up to 40% of patients with allergic rhinitis also have asthma, and up to 80% of patients with asthma experience nasal symptoms. Furthermore, patients with allergic rhinitis are at three times the risk of developing asthma compared with those without allergic rhinitis. In children who develop rhinitis within the first year of life the chance of developing asthma are twofold greater as compared with those who develop rhinitis later in life (Settipane et al., 1994; Wright et al., 1994).

Traditionally, allergic rhinitis is classified as seasonal or perennial, and as either mild, moderate, or severe. Mild allergic rhinitis involves no sleep interruption, no impairment of daily activities, and no troublesome symptoms. Moderate-to-severe allergic rhinitis involves one or more of those factors. A newer classification system specifies that allergic rhinitis be characterized as intermittent, or persistent. Intermittent disease involves symptoms for fewer than 4 days per week, or for a duration of fewer than 4 weeks. Persistent disease involves symptoms that occur more than 4 days per week and are present for longer than 4 weeks (Noble 1995; Bousquet et al., 2001). With seasonal rhinitis, the symptoms are periodic occurring in a temporal relationship to the presence of seasonal allergens in individuals who are appropriately sensitized. Pollens
causing seasonal allergic rhinitis are tree pollen present in the springtime, grass pollen present in May through July, and weed pollen and mould spores which may produce symptoms in late summer and autumn. Perennial disease, which is present all year round, relates to the presence of a non-seasonal allergen. The allergens causing perennial rhinitis are frequently indoor aeroallergens the most commonly being allergens from mites (25%; Dermatophagoides pteronyssinus/farinae), animal antigens (15%; cats, dogs, rodents), fungal spores (10%; Alternaria, Cladosporium, Aspergillus, Penicillium), or exposate to antigens at the workplace (Platts-Milis et al., 1987; Raab et al., 1989; Howarth & Holgate 1990; Noble 1995).

Allergic rhinitis should be differentially diagnosed from other respiratory allergic diseases. The two nasal conditions most commonly confused with allergic rhinitis are infectious rhinitis and perennial nonallergic rhinitis (vasomotor rhinitis). Infectious rhinitis is characterized by constitutional symptoms and purulent rhinorrhea. A nasal smear shows a preponderance of neutrophils, whereas in allergic rhinitis, eosinophils predominate. Perennial nonallergic rhinitis is more frequent in women and is precipitated by such nonspecific factors as changes in temperature, humidity, and barometric pressure; strong odours; alcohol; and cigarette smoke. Nasal congestion frequently shifts from side to side and is often alleviated by exercise (Zeiger 1989).

1.0.2 Rhinitis in common laboratory animals

Allergic diseases are very uncommon in the animal world. There is no animal species that suffers spontaneously from allergic rhinitis (Szelenyi 2000). However, the
disease can be induced in animals using different strategies. Although, different laboratory animal species have been used for the establishment of animal models of allergic rhinitis, using a variety of antigens, there is no animal model that mimics all of the symptoms of allergic rhinitis. Therefore, only single symptoms are normally induced in a certain animal species. Among the species, guinea pigs have recently gained more attention as the most suitable experimental model for in vivo studies of pharmacological and pathophysiological aspects of the acute and chronic phases of allergic rhinitis. In addition, BALB/c mice have been utilized mostly for immunological studies in allergic rhinitis (Saito et al., 2002; Murasugi et al., 2005). Furthermore, Brown Norway rats are also used for studying drugs effects on symptoms of experimental rhinitis (Sugimoto et al., 2000b; Shimizu et al., 2000; Fu et al., 2003). Among other species, dogs and pigs have been occasionally used for studies of mucus secretions and nasal congestion after allergen challenge (Revington et al., 1997; Szelenyi et al., 2000; Malis et al., 2001; Tiniakov et al., 2003).

Since this thesis concerns studies in guinea pigs, rhinitis is considered in detail for this species.

1.0.3 Rhinitis in guinea pigs

Conventionally, guinea pigs have been the species of choice for the evaluation of chemical-related respiratory allergy, primarily because it is possible in this species to elicit and measure with relative ease challenged-induced inflammatory reactions that resemble in some ways the acute clinical manifestations of human allergic rhinitis. Dunken Hartley guinea pigs have been widely used to evaluate the actions of drugs and
their therapeutic applicability in allergic rhinitis. The disease can be induced in guinea pigs using different allergens. The process of induction of allergy requires an initial sensitization dose of allergen, followed by repeated booster doses, and finally a challenge dose. Symptoms and signs of allergic rhinitis including sneezing, nose rubbing, rhinorrhea, vascular permeability (exudation) and nasal congestion. Biochemical and cellular changes can be quantified and evaluated after a challenge dose.

Ovalbumin and Japanese cedar pollen are the two common used allergens used to sensitize guinea pigs. The techniques used range from injection (intraperitoneal, with or without, adjuvant), bolus instillation (intranasal) or steady-state inhalation exposures over short or long periods of time with different concentrations of allergen and adjuvant. In the purely nasal route for sensitization, 4% lidocaine is insufflated over a period of five minutes followed by intranasal doses of allergen absorbed out in aluminum hydroxide, given daily for seven days (Mizutani et al., 1999; Yamasaki et al., 2001; Nabe et al., 2001; Fukuda et al., 2003; Zhao et al., 2005). Alternatively, guinea pigs can be exposed to 1% aerosol ovalbumin twice for ten minutes, a week apart (Yamasaki et al., 1997). Moreover, ovalbumin absorbed in aluminum hydroxide can be injected peritoneally as an initial sensitization dose (Namimatsu et al., 1991; Narita et al., 1998; Fujita et al., 1999; Imai et al., 2000; Mcleod 2002; Sakairi 2005). Guinea pigs can also be sensitized passively to ovalbumin by intravenous, subcutaneous, or intraperitoneal administration of anti-OVA serum (Mizuno et al., 1991; Kaise et al., 1999; Kaise et al., 2001a).

Following initial sensitization dose, guinea pigs are either exposed to repeated doses of allergen boosters, over a period of time, or directly challenged with allergen. The waiting period from first sensitization dose to first challenge dose varies between
studies, ranging between two weeks to more than four weeks. Conscious guinea pigs are challenged by the intranasal route using inhalation of aerosolized allergen or by instillation of micro milliliters of allergen absorbed in saline, whereas anaesthetized animals are challenged through either intranasal route or from the tracheal side toward the nasal cavity by infusion of high volumes of allergen (Albert et al., 1998; Mizutani et al., 1999; Sakairi 2005).

1.1 The structure and function of the normal human nose compared with those of common laboratory animals

1.1.1 Anatomy and Histology

The human external nose surrounds the nostrils and one-third of the nasal cavity, which in its entirety consists of a 5-cm high and 10-cm long dual chambers. The total surface area of both nasal cavities is about 150 cm\(^2\), and total volume about 15 ml. Approximately 1.5 cm from the nares is the narrowest portion of the entire airway, the internal ostium (or nasal valve), with a cross-sectional area of about 30 mm\(^2\) on each side. The nasal valve accounts for approximately 50% of the total resistance to respiratory airflow from the nostril to the alveoli (Baroody 1997). Each of the two nasal cavities is limited by the septal wall and the lateral wall, dominated by inferior, middle and superior turbinates. They are important for maintaining a slit-like cavity, and thereby facilitate humidification and temperature regulation of the inspired air (Niels et al., 1998).
The nostrils are covered by skin, the anterior one-third of the nasal cavity by a squamous and transitional epithelium. The upper part of the cavity is covered by an olfactory epithelium and the remaining portion by a typical airway epithelium, which is ciliated, pseudostratified, and columnar. The latter consists of four major cell types, basal cells, ciliated and non-ciliated columnar cells, and goblet cells. Basal cells, which are progenitors of the other cell types, lie on the basement membrane and do not directly have contact with the airway lumen (Evans et al., 1988). Each of the columnar cells, ciliated and non-ciliated cells, are covered by about 300 microvilli uniformly distributed over the entire apical surface. These short and slender fingerlike cytoplasmic expansions increase the surface area of the epithelial cells, thus promoting exchange processes across the epithelium. The microvilli also prevent drying by retaining moisture essential for ciliary function. The cilia have a typical ultrastructure, each ciliated cell containing about 100 cilia, 0.3 μm wide and 5 μm in length (Halama et al., 1990). The anterior one-third of the nasal cavity is non-ciliated. Cilia start occurring just behind the front edge of the inferior turbinate, and cover the posterior part of the nasal cavity. The paranasal sinuses, are densely covered by cilia. The distribution pattern of ciliated cells corresponds well with the distribution of nasal airflow, thus the density of ciliated cells is inversely proportional to the linear velocity of inspiratory air in the nasal cavity (Cole 1982). Another cell type characteristic of airway epithelium is the goblet cell. The majority of goblet cells are located in the posterior part of the nasal cavity with the mean concentration of 4000–7000 cells per mm² (Tos 1983). Goblet cells produce small amounts of viscous mucus that contributes only little to the total volume of nasal
secretion. Little is known about release mechanisms for goblet cells, which in contrast to the glands, are not under the control of the parasympathetic nervous system.

The glands in the nose are of two types: anterior serous glands and seromucous glands. There are only 100–150 anterior serous glands on each side of the nose. Their long excretory ducts have large openings in the upper part of the internal ostium, where small droplets of watery secretion can be seen after stimulation of the nasal mucosa. Secretions produced in the anterior part of the nose are more watery, have a considerably lower viscoelasticity than secretions produced in the posterior part of the nose (Brofeldt et al., 1979). There are about 100,000 seromucous glands in the human nose, and this number appears to remain constant during life (Tos 1983). Thus infants have a secretory capacity comparable to that of an adult. Since the ciliated surface, however, is much smaller in children, one can imagine that slight glandular hypersecretion may result in nasal discharge in the child, but not in the adult. Figure 1.1 describes the arrangement of nasal mucosa layers.

1.1.2 Vasculature

Blood from the ophthalmic and internal maxillary arteries feeds a huge network of arterioles, venules, capillaries, capacitance vessels and shunt vessels. Together these supply and drain the nasal mucosa with a greater blood flow per volume of tissue than the liver or brain (Grevers et al., 1996). The arterioles are conspicuous in an absence of an internal elastic membrane such that the endothelial basement membrane is continuous with the basement membrane system of the smooth muscle cell (Cauna 1970). The capillaries, just below the surface epithelium and surrounding the glands, are of the
fenestrated type. Thus these capillaries are well suited for rapid movement of fluid through the vascular wall allowing water to escape into the airway lumen, and for evaporation to take place so as to condition (humidify) inspired air (Cauna et al., 1969). Large venous cavernous sinusoids, mainly localized to the inferior turbinates, are characteristic of the nasal mucous membrane. They are normally found in a semi-contracted condition resulting from sympathetic nerve-mediated smooth muscle tone. The cavernous sinusoids are regarded as specialized vessels adapted to the functional demands of the nasal airway with respect to heating and humidification of inhaled air. When they distend with blood the mucosa will swell and tend to block the airway lumen (Niels et al., 1998). Furthermore, extravasation takes place through the walls of postcapillary venules during inflammation of the mucosa, by the opening of gaps in the intercellular junctions between endothelial cells (Cauna 1970).

Blood can bypass the capillary bed via arteriovenous anastomoses. The role of the arteriovenous anastomoses is probably related to temperature and water control. At least 50% of the blood flow in the nasal mucosa is normally shunted through arteriovenous anastomoses and total blood flow per cm³ of tissue is greater in the upper airway mucosa than in muscle, brain and liver (Anggard 1974; Drettner et al., 1974).

Nasal blood vessels are under endothelial and neuronal control (Riederer et al., 2002). A dual (endothelial and neuronal) control exists in arterioles whereas the control in the subendothelial muscular swellings of the cushion veins appears to be mainly neuronal.
The swelling of the nasal mucosa is achieved by a simultaneous relaxation of all smooth muscle cells, which leads to dilatation of arteries as well as venous sinuses. The drainage of the vascular bed is reduced by the venous muscular bolsters protruding into the lumen of the venous sinuses. Vice versa, a contraction of all smooth muscle cells leads to a contraction of the arteries and, consecutively, to a reduction of blood supply. Simultaneously the muscular bolsters are rise out of the lumen of venous sinusoids allowing blood drainage to be increased hence nasal decongestion (Figure 1.2).
1.1.3 Innervation

The nasal mucosa, including glands and blood vessels, are supplied by both afferent and efferent neurons (Figure 1.3). The afferent neuronal supply can be divided into two parts: the first, the olfactory nerve (cranial nerve I), projects into the olfactory mucosa, and conducts the sensation of smell; the second, cranial nerve V, projects to the epithelium and detects perception of airflow via A fibres, and noxious stimuli via unmyelinated C fibres and A\(\sigma\) fibres. Activation of these afferent nerves leads to local axonal and central reflexes (Baraniuk 1998).
Central nervous system

Trigeminal ganglion

Sphenopalatine (parasympathetic ganglion)

Nasal congestion, Plasma exudation, Inflammation, Mucus secretion

Superior cervical sympathetic ganglion

Preganglionic

Postganglionic

Sneezing

Itching

Figure 1.3: Schematic representation of the innervation of the nose. NANC: non adrenergic non cholinergic, CGRP: calcitonin gene related peptide, Sub-P: substance P, NK-A: neurokinin A, ACh: acetylcholine, VIP: vasoactive intestinal peptide, NO: nitric oxide, NEP: norepinephrine, NPY: neuropeptide Y.
There is a rich parasympathetic innervation to glands. Nervous stimulation of glandular cholinoceptors causes marked hypersecretion and is often part of a reflex arc. Blood vessels, have both sympathetic and parasympathetic innervation, but are controlled mainly by sympathetic fibres. A continuous release of noradrenaline is postulated to keeps the sinusoids partly contracted since vasoconstrictor effect of stimulation of the alpha- adrenoceptor is more marked than vasodilatation resulting from stimulation of the $\beta_2$-receptor (Niels et al., 1998).

The classical neurotransmitters, noradrenaline and acetylcholine, have in recent years been found to be accompanied by a number of peptide neurotransmitters. These are secreted by afferent unmyelinated C fibres (substance P, calcitonin gene-related peptide (CGRP), neurokinin A (NKA), gastrin-releasing peptide), from efferent parasympathetic nerve endings (vasoactive intestinal peptide (VIP), peptide histidine methionine), and from efferent sympathetic nerve endings (neuropeptide Y) (Uddman et al., 1987; Lundblad 1990; Baroody 1997). Neuropeptides are capable of generating local reflexes which causes an increase in vascular permeability, plasma leakage, vasodilatation and subsequent tissue oedema (Baraniuk 1997).

1.1.4 Physiological function

Apart from being the first part of the airways, the nose has two major functions; firstly, olfaction, and secondly, conditioning of the inspired air for the lungs, by heating, humidifying and cleansing inhaled air. The normal nose is characterized by slit-like passages, which provide for efficient exchange of heat and moisture and the width of these nasal cavities are actively regulated via the sympathetic innervation and tone in the
venous sinusoids. Nasal cycling is the cyclic alteration between the resistances on the two sides of the nose. This changes from one side to the other at 2–4-h intervals. Eighty percent of humans show this nasal cycle, and it has also been demonstrated in rat, rabbit, and pig. In addition, the nasal cycle is perceived by subjects with a deflected septum and by rhinitis patients (Niels et al., 1998). The nasal cycle seems to be predominantly vascular, and it is mediated via the nervous control of the sinusoidal erectile tissue. Cutting the cervical sympathetic nerves, or blocking the sympathetic supply by local anesthesia, abolishes the nasal cycle in human and in lower animals (Widdicombe, 1986).

The nose is well suited to its air-conditioning function: (i) the slit-like shape of the nasal cavity assures close contact between the inhaled air and the mucous membranes; (ii) the width of the cavity can adapt rapidly to changing needs by alteration in sinusoid contraction; (iii) heat exchange is facilitated by the large amount of arterial blood flowing in arteriovenous anastomoses, analogous to hot water in a radiator; (iv) the nasal mucosa has a high secretory capacity. Furthermore, the body saves about 100 ml of water per day, due to condensation of exhaled water in the anterior part of the nose, which has a temperature 3–4°C lower than that of the lungs. This water may contribute to rhinorrhea in cold weather.

In addition the nose acts as a filter. Almost all particles larger than 10 μm (e.g. pollen grains) are retained in the nose during breathing at rest, while most particles smaller than 2 μm (mould spores) can bypass the nose. The nose also acts as a protective filter for water-soluble gases (sulphur dioxide, formaldehyde). Inhaled particles, trapped
in the nasal filter, are cleared from the nose within 30 min by mucociliary transport (Hilding et al., 1963; Andersen et al., 1974).

1.1.5 Nose structure and function of other species

In addition to the obviously wide range of size and external shapes of the nose between human and animals, there are also clear interspecies differences in the internal anatomy and physiology of the nose (Figure 1.4).

Figure 1.4: External and internal anatomical arrangement of human and guinea pig noses. A and B indicate external appearance of human and guinea pig noses respectively. C and D show nasal cavities of human and guinea pig respectively. Composite from non-copywrite web sources.
The development of the nasal cavity in most mammals, excluding man and some higher apes (orangutan, chimpanzee, gorilla), is reflected in its primary function of olfaction. Carnivores including dog and cat, and other species, such as rodents have complex nasal cavities with large areas for olfaction. On the other hand, the fact that optimum temperature and humidity are necessary for the detection of odor and normal function of the lower respiratory tract, plus the characteristic rapid movements and breathing patterns of these animals, has resulted in the development of a relatively large surface area for air conditioning. This air conditioning mechanism is especially apparent in desert animals, such as the camel, and in cold-water diving mammals such as seals. Thus, from a comparative viewpoint, humans have relatively simple noses with the primary function for breathing, while other mammals have more complex noses with primary function for olfaction. As a result of this distinction in primary function, the anatomy of the nasal cavity in relation to the oral cavity is arranged in such a manner that, while man (and some higher apes) can breathe both nasally and oronasally, other mammals are generally obligatory nose breathers, due to the close apposition of the epiglottis to the soft palate in such species (Proctor et al., 1983).

Despite the greater complexity and the variations in the shape and dimension of the nasal cavity and its turbinates, the nasal airways of most animals have characteristics similar to those in humans. From nostrils, which are in line with the nasal fossae in most instances, air must pass the vestibule and through the nasal valve into the main chamber (nasal cavity) that is divided into two rather symmetrical compartments. Posterior to the termination of the nasal septum, the air passages then merge into one and travel downward through the nasopharyngeal meatus into the nasopharynx. Beyond their
similarity in airway anatomy, there are major structural differences between man and other mammals in the nostrils, vestibules, nasal septum, and the turbinates that can modify the course of the air current. In some diving mammals the nostrils can be regulated to open and close, while those of others are comma-shaped. The vestibule of rats, mice and cats contains atrioturbinates that are effective baffle systems to deflect a large volume of air and trap particulates and contain lateral nasal glands which are absent in man. The septum of rats, mice, hamsters, and guinea pigs contains the so-called "septal window: so that in some experiments, the two halves of the chamber cannot be treated individually (Kelemen 1950; Kelemen 1953; Bang et al., 1959).

Histologically, the relative distribution of squamous, respiratory, and olfactory epithelium from nostrils to nasopharynx is similar between man and other mammals although the nasal respiratory epithelium of man appears to be more evenly covered by cilia, goblet cells and secretory acini than most animals (Negus 1959).

Underneath the respiratory epithelium, the lamina propria is rich in venous plexes. When these are altered, they can affect the thickness of the respiratory mucosa and thus the width of the airway. In general, there is very little difference between man and other mammals in the organization and the ultrastructure of the vascular system within the nose (Dawes et al., 1953; Van Diest et al., 1979). The choncae of guinea pig nose has extensive network of arterioles and venules. The arterioles and the venules are richly innervated, abounding in cholinergic nerve endings with peptidergic vesicles. The glands are located in the posterior portion and are composed of acini, and intercalated
and striated duct. Acini as well as the intercalated duct have cholinergic nerve endings and vesicles, but also a probable peptidergic produce (Pastor et al., 1990).

In guinea pig nose, the distribution of fibers containing vasoactive intestinal polypeptide (VIP) is dense in the glandular tissue, but sparse around blood vessels and very sparse in the subepithelial layer. Fibers containing calcitonin gene-related peptide (CGRP) are densely distributed around blood vessels, glandular tissue and the subepithelial layer. A moderate number of CGRP containing fibers were observed in the intraepithelial layer. Fibers containing substance P and neurokinin A are sparsely distributed around blood vessels, glandular tissue and the subepithelial layer. In addition, a greater content of both neurokinin A and substance P is located in the nasal concha than in the nasal septum (Su 1989).

1.2 Pathophysiology of allergic rhinitis in various species including human

The allergic sensitization that characterizes allergic rhinitis has a strong genetic component. The tendency to develop IgE/mast cell/TH2 lymphocyte immune responses is inherited in atopic patients. In addition, the hygiene hypothesis, first proposed by Strachan in 1989, explains the increasing prevalence of atopic conditions like allergic rhinitis (Strachan et al., 1989). The idea arose from epidemiological observations suggesting an inverse correlation between family size and the prevalence of allergic rhinitis. The hypothesis proposes that reduced contact with microbes, and diminished burden of infectious disease at an early age, leads to weakened immunological drive in the Th1 direction resulting in overactivity of Th2 responsiveness. However, substantial
evidence suggests a negative relationship between infection and atopic diseases (Li et al., 2003).

Exposure to threshold concentrations of dust mite fecal proteins, cockroach allergen, cat, dog, and other danders, pollen grains, or other allergens for prolonged periods of time leads to the presentation of the allergen by antigen presenting cells to CD4+ T lymphocytes, which then release interleukin (IL)-3, IL-4, IL-5, and other TH2 cytokines. These cytokines drive proinflammatory processes, such as IgE production, against these allergens through the mucosal infiltration and actions of plasma cells, mast cells, and eosinophils. Once the patient has become sensitized to allergens, subsequent exposures trigger a cascade of events that result in the symptoms of allergic rhinitis.

Allergic rhinitis is characterized by a two-phase allergic reaction: an initial sensitization phase where allergen exposure results in IgE formation as well as induction of the humoral response, and subsequent clinical disease after repeated antigen exposure. The clinical phase can also be further subdivided into early- and late-phase responses.

1.2.1 Sensitization

The first step towards generation of a T helper lymphocyte response is the recognition and uptake of antigen by antigen-presenting cells (e.g. dendritic cells, macrophages, B cells) that have the capacity to digest the antigen into short peptides that associate with major histocompatibility complex (MHC) molecules and to provide co-stimulation for naive T cells (Lambrecht 2001). Dendritic cells have been identified as the most effective antigen presenting cells for inducing and regulating the primary immune response in vivo and in vitro (Banchereau et al., 2000). The mucosa of the nose
is covered with an extensive network of dendritic cells which reside in the para and intercellular channels surrounding the basal epithelial cells (Evans et al., 2000).

There are three dominant mechanisms by which immature dendritic cells can uptake an antigen. First, antigenic material can be acquired via receptor-mediated endocytosis involving clathrin-coated pits. Immature dendritic cells express a plethora of specialized cell receptors for patterns associated with foreign antigens, such as the C-type lectin carbohydrate receptors (Mahnke et al., 1999; Valladeau et al., 2000; Geijtenbeek et al., 2000; Ariizumi et al., 2000; Cochand et al., 2000). Secondly, an antigen can be taken up by a constitutive macropinocytosis that involves the actin skeleton-driven engulfment of large amounts of fluid and solutes (approximately one cell volume/hour) by the ruffling membrane of the dendritic cell followed by concentration of soluble antigen in the endocytic compartment (de Baey et al., 2000). Thirdly, dendritic cells have been shown to phagocytose particulate antigens such as latex beads, and even whole bacteria, as well as apoptotic cells. This could be the dominant mechanism of uptake of particulate allergens (Banchereau et al., 2000). After being taken up by any of the above mechanisms antigens accumulate in the endocytic compartment, where they are loaded on newly synthesized and recycling MHC class II molecules. However, they may also be transported into the cytosol, where they become accessible to the class I antigen presentation pathway (Rodriguez et al., 1999; de Baey et al., 2000). Within the endocytic compartment, antigen is cleaved into short immunogenic peptides by proteolytic enzymes. Antigen is loaded on MHC class II molecules in an acidic cellular compartment rich in newly synthesized MHC class II molecules, called the MIIC compartment (Nijman et al., 1995). Alternatively, immunogenic peptides can be loaded onto pre-
formed MHC II molecules that have been internalized into mildly acidic endosomal vesicles after being expressed on the cell-surface (Cella et al., 1997). In addition, antigen processing by proteases can occur extracellularly generating peptides that can be loaded onto empty cell surface-expressed MHC class II. Surprisingly, proteolysis of antigen by immature dendritic cells can also occur extracellularly through secreted proteases, resulting in the generation of peptides that can be loaded onto empty cell surface-expressed MHC class II (Santambrogio et al., 1999). Subsequently, dendritic cells migrate through submucosa and present the processed antigen to naïve undifferentiated T<sub>Helper</sub> (T<sub>H</sub>) lymphocytes. Antigen-specific T cells bind the dendritic cell MHC class II-peptide complex with CD4 and this interaction, along with other cell-cell signals, triggers the T cells to differentiate into T<sub>H2</sub> cells and activation of B lymphocytes which produce antigen-specific IgE.

IgE is the principal trigger for allergic rhinitis. IgE interacts with both FceR1 and the lower-affinity receptor FceR2 (CD23). Differentiation of B cells into IgE-secreting plasma cells requires at least two distinct signals in IL-4 (or IL-13) and CD40L on the surface of T<sub>H2</sub> cells with CD40, a co-stimulatory molecule on B cells which triggers isotype switching to IgE. IgE binds to the α-chain of the tetrameric FceR complex on mast cells, basophils, monocytes and dendritic cells. The molecular interactions responsible for high-affinity binding are complex and involve several sites in the C<sub>E</sub>3 domain of IgE (Chang 2000).

In its free form, IgE has a half life of only a few days, however, when bound by FcεRs it is protected against degradation and can remain on the surface of inflammatory cell for months (Brostoff et al, 1996). Circulating antigen-specific IgE binds (using Fc
region) to FceRI receptors on the surface of nasal mast cells and basophils exposing the antigen-specific Fab region to the local environment, ready to be activated by further allergen exposure.

The initial exposures and the process of priming the inflammatory cells for response to antigen is referred to as sensitization. Re-exposure to the same allergen on a mucosal surface, results in a coupling or cross-linking of the IgE molecule that leads to cellular degranulation and the release of inflammatory mediators, a process resulting in both an acute and a chronic phases (Figure 1.5).

![Figure 1.5: Schematic representation of pathophysiology of allergic rhinitis. Ag: antigen, IgE: immunoglobulin E, IL: interleukin, TNF-α: tumor necrosis factor-alpha, PAF: platelet activating factor, ECP: eosinophil chemotactic protein, LTB4: leukotriene B4, GM-CSF: granulocyte macrophage colony stimulating factor, VCAM-1: vascular cell adhesion molecule 1, VLA-4: very late antigen 4, EP: epithelium, RANTES: regulated upon activation normal T cell expressed and secreted. After Pawankar 2001](image-url)
1.2.2 Acute phase

During periods of continuous allergen exposure increasing numbers of IgE-coated mast cells traverse the epithelium, recognize the mucosally deposited allergen, and degranulate (Naclerio 1991a). Products of this degranulation include preformed mediators such as histamine, tryptase (mast cell specific marker), chymase ("connective tissue"-mast cells only), kininogenase (generates bradykinin), heparin, and other enzymes. In addition, mast cells secrete several inflammatory mediators de novo (ie, one that are not preformed and stored in mast cell granules) including prostaglandin D2 and the sulfidopeptidyl leukotrienes (LT)LTC4, LTD4, and LTE4. These mediators cause blood vessels to leak and produce mucosal edema plus a watery rhinorrhea characteristic of allergic rhinitis. Glands secrete mucoglycoconjugates and antimicrobial compounds and dilate blood vessels to cause sinusoidal filling and a resulting occlusion and congestion of nasal air passages. These mediators also stimulate sensory nerves, which convey the sensations of nasal itch and congestion, and recruit systemic reflexes such as sneezing. The above responses develop within minutes of allergen exposure and are termed the early phase, or "immediate," allergic response (Mygind et al., 1993). Sneezing, itching, and copious clear rhinorrhea are characteristic symptoms during the early phase of allergic responses, although nasal congestion may also occur.

1.2.3 Chronic phase

Mast cell-derived mediators released during early phase responses, as well as mediators released by basophils during the late phase, are hypothesized to act on postcapillary endothelial cells to promote the expression of vascular cell adhesion
molecule and E-selectin which facilitate the adhesion of circulating leukocytes to the endothelial cells. Chemoattractant cytokines such as IL-5 promote the infiltration of the mucosa with eosinophils, neutrophils, and basophils, T lymphocytes, and macrophages (Naclerio et al., 1985; Bascom 1988). During the 4- to 8-hour period after allergen exposure, these cells become activated and release inflammatory mediators which in turn reactivate many of the proinflammatory reactions of the immediate response. This cellular-driven late inflammatory reaction is termed the “late phase response.” This reaction is clinically indistinguishable from the immediate reaction, but congestion tends to predominate (Skoner et al., 1988). Eosinophil-derived mediators such as major basic protein, eosinophil cationic protein, and leukotrienes have been shown to damage the epithelium, leading ultimately to the clinical and histological picture of chronic allergic disease. Subsets of the T-helper lymphocytes likely orchestrate the chronic inflammatory response to allergens. TH2 lymphocytes promote the allergic response by releasing IL-3, IL-4, IL-5, and other cytokines that promote IgE production, eosinophil chemoattraction and survival in tissues, and mast cell recruitment (Durham et al., 1992). Cytokines released from TH2 lymphocytes and other cells may circulate to the hypothalamus and result in the fatigue, malaise, irritability, and neurocognitive deficits that commonly are noted in patients with allergic rhinitis (Sim et al., 1995).

1.2.4 Inflammatory cells in allergic rhinitis

One of the hallmarks of allergic diseases is an intense accumulation of inflammatory cells in tissue locations at specific mucosal surfaces. The presence of an increased number of mast cells, basophils, T cells, and particularly eosinophils, has been
detected in nasal smears and biopsies from patients with allergic rhinitis. It has also been shown in response to certain mediators, that these inflammatory cells undergo local activation, releasing their own mediators, thereby contributing to the pathological features of the disease.

1.2.4.1 Mast cells

Mast cells are constitutive cells within the normal nasal mucosa and are the recognized key cells for type 1 hypersensitivity reactions. These cells can be subdivided into connective and mucosal phenotypes. Connective tissue mast cells express chymase, tryptase and TNF-α (Bradding et al., 1995). This cell population represents 85% of the IL4 positive mast cells in the nasal lamina propria. During allergen exposure, there is an increase in the proportion of mast cells in the epithelial cell layer (Juluisson et al., 1995). These cells which produce predominantly tryptase, without chymase, are called mucosal mast cells and are 15% of the IL4 positive mast cells.

In sensitized individuals, the nasal mucosa is full of IgE-binding mast cells (Enerback et al., 1986). Mast cells have long been considered to primarily serve as important effector cells for acute IgE-associated allergic reactions. Mast cells in patients with allergic rhinitis produce Th2 type cytokines, induce IgE synthesis in B cells, and can autoactivate via the mast cell-IgE-FcεRI cascade. In addition, mast cells upregulate the production of a variety of cytokines/chemokines in epithelial cells and fibroblasts and induce the recruitment of basophils, T cells and eosinophils into sites of allergic inflammation as well as their own intraepithelial accumulation by upregulation of adhesion molecules like VCAM-1 and through the interactions of nasal mast cells with the extracellular matrix proteins, and nasal epithelial cells. Thus, it is increasingly evident
that mast cells are not only important for the genesis of the allergic reaction, but also contribute to the late-phase allergic reaction, and to on-going allergic inflammation (Yamagishi et al. 2000; Pawankar 2005).

Activation of mast cells can occur by antigen and immunoglobulin E (IgE) via the high-affinity receptor (FceRI) for IgE. The liberation of proteases, leukotrienes, lipid mediators, and histamine contribute to tissue inflammation and allow recruitment of inflammatory cells to tissue. In addition, the synthesis and expression of a plethora of cytokines and chemokines (such as granulocyte-macrophage colony-stimulating factor [GM-CSF], interleukin-1 [IL-1], IL-3, IL-5, tumor necrosis factor-alpha [TNF-alpha], and the chemokines IL-8, regulated upon activation normal T cell expressed and secreted [RANTES], monocyte chemotactic protein-1 [MCP-1], and eotaxin) by mast cells can influence leukocytes biology which has a great effect in allergic inflammation (Shakoory et al., 2004).

1.2.4.2 Eosinophils

The eosinophil, a granular bi-lobed leukocyte readily stained by eosin (eosinophil = eosin + philein, to love in Greek), comprises approximately 2 to 5% of granulocytes in a nonallergic person. Eosinophil progenitors are released from the bone marrow into the circulation and are chemically attracted to their site of action by chemotactic factors. The development and maturation of eosinophils can also occur in situ in peripheral sites of inflammation containing pre-existing increased tissue eosinophils (Adamko et al., 2004). Activated eosinophils play a role in allergy, asthma, parasitic diseases, granulomatous disorders, fibrotic conditions and several malignant tumors (Munitz et al., 2004).
Immunohistochemical staining of nasal mucosa biopsies has show that eosinophils are evident within the submucosa and epithelium in symptomatic rhinitis (Bentley et al., 1992, Bradding et al., 1993). Eosinophils are mainly involved in the late-phase reaction after infiltration from the peripheral blood into the tissue. Cytokines secreted by Th-2 cells account for recruiting and activating eosinophils in the nose. Among them, IL-4 is considered to be pivotal since it up-regulates adhesion molecules selective for eosinophil recruitment (Krouse et al., 2002; Ciprandi et al., 2004). Eosinophils contain granules composed of four basic proteins. The core of these granules is major basic protein (MBP), while the matrix surrounding the core is composed of eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO) (Gleich et al., 1994). The possible roles of these proteins in allergic airway disease are summarized in Table 1.1. The levels of ECP, EPO and MBP are raised following antigen challenge in allergic rhinitis (Knani et al., 1992; Shin et al., 1994; Nishioka et al., 1995). Another mechanism by which eosinophils stimulate the late-phase allergic inflammation is by releasing arachidonic acid metabolites such as prostglandins and leukotrienes (Krouse et al., 2002; Saito et al., 2004). Additionally, it has been shown that human eosinophils express and synthesize a number of cytokines, including GMCSF, IL-6, IL-1α, IL-2, IL-3, IL-4, IL5, IL-8, RANTES, and TNF-α (Moqbel et al., 1991; Hamid et al., 1992; Costa et al., 1993).
Table 1.1: Role of eosinophil cationic proteins in allergic airway disease. MBP: major basic protein, ECP: eosinophil cationic protein, EDN: eosinophil-derived neurotoxin, EPO: eosinophil peroxidise.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cell content (μg/10⁶ cells)</th>
<th>Role in allergic airway disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP</td>
<td>9</td>
<td>- Histamine release from basophils and mast cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Cytotoxic to epithelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Causes bronchoconstriction and induces hyperresponsiveness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Activates neutrophils</td>
</tr>
<tr>
<td>ECP</td>
<td>5</td>
<td>- Histamine release from mast cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Cytotoxic to variety of cells</td>
</tr>
<tr>
<td>EDN</td>
<td>3</td>
<td>Undefined</td>
</tr>
<tr>
<td>EPO</td>
<td>12</td>
<td>- Cytotoxic to airway epithelium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Causes bronchoconstriction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Inactives leukotrienes</td>
</tr>
</tbody>
</table>

It is clear that eosinophils are major participants in the immunopathogenesis of allergic inflammation since they are characteristically recruited to such sites to release their cationic proteins, cytokines, and lipid mediators. Thus, they contribute to damage and dysfunction of other resident cell types and influence the inflammatory process.

1.2.4.3 Basophils

Basophils are only present in very low numbers in peripheral blood, and are not found in normal non-inflamed tissues, indicating they are recruited to sites of inflammation by mediators from other cell types. Basophils are evident in nasal smears in allergic rhinitis (Okuda et al., 1985, Otsuka et al., 1985) and can be demonstrated to increase in rhinitic patients following nasal allergen challenge (Bascom et al., 1988). Evidence of basophil infiltration into the nasal mucosa during allergen challenge is based on the mediator profile in nasal secretions (Naclerio et al., 1985, Bascom et al., 1988).
Basophils, like mast cells, possess high affinity IgE receptors, and are derived from CD34-positive progenitor cells in the bone marrow (Knapp 1990). When activated, basophils are prominent sources of the inflammatory mediators found in allergic late-phase reactions, such as histamine and LTC₄.

Basophils possess fewer, larger granules and differ from mast cells in that they contain less histamine. Following IgE-dependent activation, basophils only release 20-30% of the histamine released from a comparable number of mast cells (Cantells et al., 1987). Human basophils have also been shown to secrete cytokines, particularly IL4 and IL-13, when activated by IgE-dependent stimuli, modulating their response and the immune responses of other cell types that participate in allergic rhinitis (Mac Glashan et al., 1994, Schroeder et al., 1994, Schroeder et al., 1996).

1.2.4.4 T lymphocytes

T lymphocytes have evolved to coordinate and amplify the effector functions of antigen specific and non-specific inflammatory cells such as B cells and eosinophils. T lymphocytes have been divided into two distinct subtypes based upon their effector functions. CD4+ T cells represent the T helper cells, which are important in the regulation of antigen-driven inflammatory processes. Via antigen-specific T cell receptors, CD4 T cells are capable of recognizing processed foreign antigen in association with MHC class II on specialized antigen-presenting cells (e.g. macrophages and dendritic cells). On the other hand, CD8+ T cells which represent T suppressor cells, drive the cell-mediated response and respond to APC presenting antigen in conjunction with MHC class II molecule. The T lymphocyte represents a significant non-structural
cell within the nasal mucosa. An increase in the release of these cells has been described in nasal biopsies specimens from rhinitic patients. These are generally CD4+ T\textsubscript{H2} cells displaying the activated phenotype (CD25+) (Varney et al., 1992; Calderon et al., 1994). CD4+ T\textsubscript{H2} lymphocytes have been shown to play a crucial role in the induction and maintenance of chronic allergic inflammation. The presence of T lymphocytes in allergic inflammation has been well demonstrated. However, the major reason for their importance lies on the profile of cytokines they express upon activation. Although individual T cells have the capacity to produce a wide range of cytokines, a restricted profile of cytokines is seen in chronic inflammatory diseases (Kelso 1995).

A major feature of allergic diseases is the high expression of Th2-type cytokines. T lymphocytes of the T\textsubscript{-H2} subpopulation can generate IL-3, IL-4, IL-5, GM-CSF and TNF-\(\alpha\) (Mossman et al., 1989). The Th2 phenotype is thought to influence subsequent T cell activation and IgE production by B cells in addition to promoting the attraction, activation, growth, and differentiation of specific leukocytes such as eosinophils. In this way, activated T cells can initiate and propagate allergic inflammation and participate directly in the events responsible for allergic diseases.

1.2.4.5 Epithelial cells

Besides being part of nasal mucosa barrier, epithelial cells have the ability to generate pro-inflammatory cytokines and chemokines that can play an important role in the genesis and persistence of allergic rhinitis. Following exposure to allergen, in-vitro nasal epithelial cells from atopic individuals are able to release significantly greater amounts of IL-1\(\beta\), IL-8, GM-CSF, TNF-\(\alpha\) and the chemokine 'regulated upon activation,
normal T cell expressed and secreted' (RANTES), as compared with nasal epithelial cells from non-atopic individuals. Nasal epithelial cells of atopic individuals, with a genetic predisposition to upper airway disease, appear to release increased amounts of pro-inflammatory cytokines with natural exposure to allergen, augmenting the release of these cytokines, thereby exacerbating the allergic response (Calderon et al., 1997).

In a preparation of epithelial-cell-conditioned medium, human upper airway epithelial cells secrete GM-CSF, while epithelial cells from inflamed nasal tissue were shown to secrete larger amounts of pro-inflammatory cytokines as compared with normal nasal epithelial cells (Ohtoshi et al., 1991). During inflammation, complement activation has been shown to occur upon the nasal epithelial cell membrane with the nasal epithelium being capable of regulating this process. The integrity of the nasal epithelium in inflammatory states is thought to depend on the maintenance of an equilibrium between complement activation and cell membrane regulation of this activation (Varsano et al., 1996).

Other cells types have also been shown to increase in allergic rhinitis including neutrophils, macrophages, dendritic cells, monocytes, B cells (Bachert et al., 1998) although their crucial role in the responses to allergen challenge is controversial.

1.2.5 Inflammatory mediators in allergic rhinitis

1.2.5.1 Histamine

Histamine plays a pivotal role in allergic inflammation. It is synthesized from L-histidine by histidine decarboxylase. Histamine is released from the granules of FceRI+
cells (e.g., mast cells and basophils), after the cross-linking of surface IgE by allergen or through mechanisms that are independent of IgE (Enerback et al., 1986; Kaliner 1994; Howarth 1995). Nasal challenge with histamine causes sneezing, pain, pruritus, rhinorrhea and nasal blockade (Doyle et al., 1990). Activation of sensory neurones by histamine causes sneezing and pruritus (Mygind 1982) in addition to activating a central reflex-mediated increase in nasal parasympathetic activity (Hilberg et al., 1995). Increased release of parasympathetic mediators (e.g. acetylcholine) stimulates nasal submucosal glands which, together with the increase in vascular permeability, cause rhinorrhea (Baroody et al., 1994).

All four histamine receptors (H1, H2, H3 and H4) have been found in the nasal mucosa by molecular biology studies (Nakaya et al., 2004), with higher expression of H1 and H2 in atotics (Iriyoshi et al., 1996; Hirata et al., 1999). Most of the effects of histamine in allergic disease occur through H1 receptors (Schmelz et al., 1997; Schneider et al., 2002; Akdis et al., 2003), whereas cutaneous itch and nasal congestion may occur through both the H1- and H3-receptors (McLeod et al., 1999; Sugimoto et al., 2004). Histamine also activates H2-receptors on the smooth muscle cells surrounding nasal capacitance vessels. They mediate muscle relaxation, increase blood content, and thereby enlarge the volume of nasal mucosa. In addition, histamine modulates function of immune cells via H4-receptors (Riechelmann 2005). In addition to its role in the early allergic response to antigen, histamine acts as a stimulatory signal for the production of cytokines and the expression of cell-adhesion molecules and class II antigens, thereby contributing to the late allergic response (Fujikura et al., 2001; MacGlashan 2003).
H₁-receptors belong to the superfamily of G-protein-coupled receptor. In addition, it has recently been shown that these receptors demonstrate agonist-independent signal transduction. H₁-antihistamines inhibit this constitutive signaling, probably by stabilizing an inactive conformation of the H₁-histamine receptor and acting as inverse agonists (Bakker et al., 2002). Via the H₁-receptor, histamine has proinflammatory activity and is involved in the development of several aspects of antigen-specific immune response, including the maturation of dendritic cells, and the modulation of the balance of type 1 helper (Th₁) T cells and type 2 helper (Th₂) T cells. Histamine may induce an increase in the proliferation of Th₁ cells and in the production of interferon gamma and may block humoral immune responses by means of this mechanism. Histamine also induces the release of proinflammatory cytokines and lysosomal enzymes from human macrophages and has the capacity to influence the activity of basophils, eosinophils, and fibroblasts (Ma et al., 2002; Akdis et al., 2003).

1.2.5.2 Eicosanoids

Eicosanoids are proinflammatory mediators resulting from metabolic degradation of the arachidonic acid originating from membrane phospholipids. They include leukotrienes, prostaglandins and thromboxanes.

1.2.5.2.1 Leukotrienes

The name leukotriene comes from the words leukocyte and triene (a compound with three double bonds). What would be later named leukotriene C, "slow reaction smooth muscle-stimulating substance" (SRS) was originally described between 1938 and
1940 by Feldberg and Kellaway. The researchers isolated SRS from lung tissue after a prolonged period following exposure to snake venom and histamine (Feldberg et al., 1938; Kellaway et al., 1940).

Leukotrienes are generated by the action of 5-lipoxygenase on arachidonic acid. They are released in both the early and late phases following antigen challenge in subjects with seasonal allergic rhinitis, and during the early phase in perennial allergic rhinitis (Naclerio et al., 1985; De Graaf-in't Veld et al., 1996). There are two classes of leukotrienes: LTB₄ and the peptidyl-cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄). Leukotrienes have important mediator functions in the upper airways, with implications for the treatment of allergic rhinitis. At least two classes of receptors exist for the cysteinyl leukotrienes and are termed cysLT1 and cysLT2 (Nicosia et al., 1999). The CysLT1 receptor is found in the human airway (including airway smooth muscle cells and airway macrophages), on other pro-inflammatory cells (including eosinophils and certain myeloid stem cells) and in nasal vascular beds. CysLTs have been intimately related to the pathophysiology of asthma and allergic rhinitis.

Leukotrienes are synthesized by inflammatory cells known to play a key role in allergic rhinitis (i.e. mast cells, eosinophils and basophils). Moreover, LTC₄ and LTD₄ are released at measurable concentrations in nasal secretions when nasal mucosa is exposed to allergen (Howarth et al., 2000). In one study, ragweed-sensitive patients challenged intranasally with pollen grains demonstrated a dose-dependent release of LTC₄, LTD₄ and LTE₄ in nasal lavage, which was correlated with the familiar symptoms of increased nasal airway pressure, sneezing, and mucous secretion (Creticos et al., 1984). Furthermore, LTC₄, LTD₄ and LTE₄ cause a long-lasting eosinophilic infiltration,
and have been associated with airway hyperresponsiveness in the lower airways in rats and in man (Christie et al., 1992; Wang et al., 1993).

Nasal challenge with CysLTs can reproduce the symptoms of allergic rhinitis, the effects of which can be inhibited by administration of leukotriene receptor antagonists. In a study in normal subjects, topical delivery of LTD\textsubscript{4} into the nose produced a significant dose-dependent increase in nasal mucosal blood flow and nasal airway pressure. Similar data have been seen in allergic subjects, whereby nasal provocation with LTD\textsubscript{4} produced a marked increase in nasal airway pressure (McLeod et al., 1988). In addition, pre-treatment with pranlukast (a leukotriene receptor antagonist) inhibits the nasal mucosal swelling induced by topical administration of LTD\textsubscript{4} (Numata et al., 1999).

1.2.5.2.2 Prostaglandins and thromboxanes

Prostaglandins are proinflammatory mediators resulting from metabolic degradation of arachidonic acid originating from membrane phospholipids. The most important products of enzyme cyclooxygenation of arachidonic acid are prostaglandins D\textsubscript{2}, E\textsubscript{2}, F\textsubscript{2α}, thromboxane A\textsubscript{2} and prostacyclin (Raskovic et al., 1998). Prostaglandins PGD\textsubscript{2} and PGE\textsubscript{2} are detected at increased levels in nasal lavage fluid following allergen challenge in subjects with seasonal allergic rhinitis (Sugimoto et al., 1994; Wagenmann et al., 1996), and perennial allergic rhinitis (Ramisi et al., 1991), but only in the early response, and not the late phase of inflammation. However, inhibitors of cyclooxygenase, the enzyme required for the synthesis of prostaglandins, do not affect the response to antigen in human nasal airways (Naclerio et al., 1985), suggesting a minimal role of prostaglandins involvement in allergic rhinitis.
On the other hand, thromboxane A2 induces vascular permeability, eosinophil infiltration in nasal mucosa and nasal congestion after antigen challenge in allergic patients. In addition, the level of nasal thromboxane A2 is also increased after antigen challenge in allergic individuals (Motobayashi et al., 2001).

1.2.5.3 Nitric oxide (NO)

NO is produced by the action of NO synthase (NOS) on the substrate L-arginine. Different isoforms of nitric oxide synthase exist: neuronal, inducible, and endothelial forms. There is evidence that NOS activity is increased in perennial allergic rhinitis (Garrelds et al., 1995) and in seasonal allergic rhinitis (Martin et al., 1996; Kharitonov et al., 1997). In mice with allergic rhinitis, the distribution of the different nitric oxide synthases in nasal mucosa was examined. Neuronal and endothelial nitric oxide synthases were found on the surface epithelial and vascular endothelial cells, with no differences between allergic mice and the control group mice. However, the amount of inducible nitric oxide synthase was elevated in allergic mice (Oh et al., 2003).

Nitric oxide may also have a role in the production of cytokines necessary for eosinophil survival, such as IL-4 and IL-5 (Barnes et al., 1995). Interestingly, NO is thought to be the main mediator of inhibitory non-cholinergic non-adrenergic (NANC) transmission. Therefore, inhibition of NOS could cause a reduction in the activity of inhibitory NANC nerves, thereby potentiating neurogenic inflammation mediated by excitatory NANC nerves. In chronic allergy, excessive NO production causes airway hyperresponsiveness via the formation of the peroxynitrite free radical in guinea pigs airways through inhibiting cGMP production (Sadeghi-Hashjin et al., 1996).
Furthermore, other NO metabolites, such as nitryl chloride, can be synthesised by neutrophils, inactivating endothelial cell angiotensin-converting enzyme (Eiserich et al., 1998). This enzyme is involved in the degradation of kinins and possibly tachykinins in allergic rhinitis (Lurie et al., 1994; Chatelain et al., 1995). Thus inhibition of this enzyme may influence nasal AHR by potentiating the action of these mediators.

1.2.5.4 Platelet activating factor (PAF)

PAF is not preformed in storage granules, but produced from phospholipids mobilized from cell membranes by phospholipase A2 in many cell types (e.g., basophils, neutrophils, monocytes, macrophages or endothelial cells). PAF produced by monocytes and polymorphonuclear leukocytes is secreted, whereas PAF synthesized by vascular endothelial cells activated by various physiologic agonists (e.g., thrombin, bradykinin, histamine, hydrogen peroxide, and leukotrienes C4 and D4) is not released (Sisson et al., 1987; Leirisalo-Repo 1994; Cuss 1999; Krump et al., 1999).

Of all the inflammatory mediators involved in allergic rhinitis, PAF is perhaps the most potent for inducing vascular leakage, an event that contributes to rhinorrhea and nasal congestion (Cuss 1999; Oppenheimer et al., 2002). PAF has potent proinflammatory properties that have been implicated in bronchial asthma (Naclerio et al., 1985). However, its role in allergic rhinitis is less well established. Several studies have been performed with PAF receptor antagonists in animal allergic rhinitis. In guinea pigs, CV-3988 blocked vascular permeability and decreased nasal airway pressure induced by topical application of PAF, whereas SM-10661 attenuated antigen-induced increase in late-phase nasal airway pressure (Honda et al., 2002). Another PAF
antagonist, ABT-491, inhibited both antigen-induced leakage and decreased airway resistance in rats and guinea pigs (Bousquet 1998). In the clinic, instillation of PAF into the nose induces many of the symptoms of rhinitis, such as an increase in nasal airway pressure, rhinorrhea, nasal neutrophil influx, and nasal hyperresponsiveness (Andersson et al., 1988; Leggieri et al., 1991; Miadonna et al., 1996). Both PAF and its metabolite (lyso-PAF) have been detected in the nasal fluids and plasma of patients with rhinitis (Labrakis-Lazanas et al., 1988; Miadonna et al., 1989; Shirasaki et al., 1990).

1.2.5.5 Cytokines

Cytokines as intercellular messenger peptides, are released by a variety of cells to influence the activity of other cells. Three cytokines are of importance in the development and regulation of eosinophil function: the interleukins IL-3 and IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF). All three prevent apoptosis and prolong the survival of eosinophils in vitro. In particular, IL-5 is essential for the differentiation of progenitor cells into eosinophils (Sanderson et al., 1993).

Both IL-4 and IL-5 have been implicated in the development of airway hyperresponsiveness (Hogan et al., 1997). In animals, IL-5 causes marked eosinophilia, eosinophil activation and airway hyperresponsiveness (Van Oosterhout et al., 1996). IL-4 regulates the activity of CD4+ T-lymphocytes, which release a range of cytokines capable of priming and activating eosinophils (Mauser et al., 1993). It also activates neutrophils (Howarth 1995). Furthermore, memory T-cells in the nasal mucosa of patients with nasal allergy can produce IL-4 during allergen exposure. This could upregulate the inflammatory response (Boey et al., 1989). Patients with seasonal allergic
rhinitis or perennial allergic rhinitis have a raised number of CD4+ T-cells (Hellquist et al., 1992).

Following nasal allergen challenge in humans, levels of IL-1alpha, IL-1beta, IL-5, IL-6, IL-8 and GM-CSF are elevated in nasal secretions (Bradding et al., 1993; Gosset et al., 1993; Sim et al., 1995). Eosinophils are potential sources of these cytokines (Lantero et al., 1996). Moreover, epithelial cells isolated from allergic rhinitic patients showed increased immunostaining for GM-CSF, IL-8, the receptors for IL-1 and TNF-alpha (Galli et al., 1994), and also they release more IL-1beta, IL-8, GM-CSF and TNF-alpha compared to epithelial cells from non-allergic subjects (Nonaka et al., 1996). Similar increases in IL-4-, IL-5- and GM-CSF-positive cells are observed in biopsies from the nasal mucosa of atopic patients (Calderon et al., 1997). Both interferon-gamma and TNF-alpha (and possibly other cytokines) cause an upregulation of ICAM-1 on human nasal epithelial cells (Durham et al., 1992) while IL-4 upregulates the expression of VCAM-1. Both these adhesion molecule are upregulated in allergic rhinitis (Bradding et al., 1993).

1.2.5.6 Chemokines

Chemokines are cytokines that possess chemotactic activity. They are divided into groups depending on their chemical structure. The two main groups are CC chemokines, where two cysteine residues are adjacent to each other (e.g. RANTES, MIP-1alpha, eotaxin) and CXC chemokines, in which the two cysteine residues are separated by a third amino acid (e.g. IL-8) (Barnes et al., 1998). The concentrations of RANTES, MIP-1alpha, eotaxin, and IL-8 detected in nasal lavage are raised following nasal allergen challenge in man (Sim et al., 1995; Rajakulasingam et al., 1997; Minshall et al.,
Mucosal cells obtained from the noses of subjects with allergic rhinitis show increased expression of mRNA for RANTES (Rajakulasingam et al., 1997), and eotaxin (Minshall et al., 1997). It is now generally accepted that RANTES and eotaxin are important in IL-5-mediated eosinophilia, where the latter causes the mobilisation of eosinophils into the circulation while the local release of chemokines provides a 'homing' mechanism for the migration of eosinophils into tissues (Barnes et al., 1998). Administration of RANTES into the nasal airway of subjects with allergic rhinitis causes an eosinophilia, but does not increase other inflammatory cells (Kuna et al., 1998). However, the same study also found that, after allergen challenge, administration of RANTES also caused an influx of basophils, neutrophils, lymphocytes and monocytes, as well as causing epithelial shedding, a response similar to that observed in nasal hyperresponsiveness. It is therefore likely that chemokines have an important role in the recruitment of inflammatory cells that is observed during the development of nasal hyperresponsiveness.

1.2.5.7 Kinins

Kinins are proinflammatory peptides that mediate numerous vascular and pain responses to tissue injury. Two pharmacologically distinct kinin receptor subtypes have been identified and characterized for these peptides, which are named B1 and B2 (Regoli et al., 1977). The B2 receptor mediates the action of bradykinin (BK) and lysyl-bradykinin (Lys-BK), whereas the B1 receptor mediates the action of des-Arg⁹-BK and Lys-des-Arg⁹-BK (Fredrik et al., 2005).
Recent investigations have found that airway hyperresponsiveness in the human nasal airway may be kinin dependent. Icatibant, a highly potent bradykinin B2 receptor antagonist, prevents PAF-induced airway hyperresponsiveness, while PAF causes an increase in the concentration of kinins in nasal lavage fluid. Kinins are produced in both perennial allergic rhinitis, and seasonal allergic rhinitis. They could therefore contribute to airway hyperresponsiveness in allergic rhinitis (Turner et al., 1999). Bradykinin causes sensitisation of C-fibres in the guinea pig trachea (Fox et al., 1996), and there is evidence that, in the human nose, enhanced responsiveness to bradykinin is mediated by neural reflexes (Riccio et al., 1996). Bradykinin can also release substance P and other neuropeptides from sensory nerve endings (Saria et al., 1988; Geppetti et al., 1990), so it may induce airway hyperresponsiveness by a neuropeptide-dependent mechanism. Alternatively, bradykinin can initiate the production of the cytokines IL-1, IL-6 and IL-8 in vivo (Ferreira et al., 1993), and stimulate the release of TNFalpha/beta and IL-1 from macrophages (Tiffany et al., 1989). In addition, it has been found that bradykinin increases the expression of the CXC chemokine receptors CXCR1 and CXCR2 in patients with allergic rhinitis (Eddleston et al., 2003), and this may contribute to nasal airway hyperresponsiveness.

1.2.5.8 Neuropeptides

Neuropeptides are contained in, and released from a wide range of nerves. Chemically distinct, they exhibit characteristic patterns of localization within the peripheral and central nervous system and possess the ability to cause a range of diverse biological responses. The nerves that contain and release neuropeptides are primarily
unmyelinated sensory C-fibres and myelinated Aδ-fibres. Such nerves provide a dense innervation to most organs and tissues, in particular, blood vessels, where perivascular nerves often terminate in close association with endothelial cells. Parasympathetic nerve endings contain vasoactive intestinal peptide (VIP), peptide histidine methionine, and efferent sympathetic nerve endings contain neuropeptide Y. Nasal sensory nerve fibres contain a number of different peptides, including calcitonin gene-related peptide (CGRP) and the tachykinins substance P and neurokinin A (NK-A). These neuropeptides, metabolised by the enzyme neutral endopeptidase (NEP), are released from sensory nerves which form part of the non-adrenergic non-cholinergic (NANC) nervous system, and are capable of generating local reflexes which causes an increase in vascular permeability, plasma leakage, vasodilatation and subsequent tissue oedema (Baraniuk 1997). Please see the section on neuronal events for details of the role of neuropeptides in allergic rhinitis.

1.2.6 Inflammatory mediators in animal models of allergic rhinitis

Laboratory animals have been used widely to study the pathophysiological changes occurring in allergic rhinitis using pharmacological, immunological and histopathological approaches. Rhinitis is induced in guinea pigs, rats, mice, dogs and pigs using different kinds of allergens with various methods of sensitization. The sensitized animals produce the different symptoms and signs of allergic rhinitis, including sneezing, nasal itching, nasal congestion, rhinorrhea. Various pro-inflammatory mediators and cells have been found to be involved in producing allergic rhinitis symptoms in the animals, mimicking what has been found in humans. Tables 1.2-1.6 summaries inflammatory
mediators and cells, and their role in producing symptoms and pathophysiological changes in allergic rhinitis in various experimental species.

Table 1.2: Inflammatory cells and mediators in guinea pig allergic rhinitis

<table>
<thead>
<tr>
<th>Mediators and cells</th>
<th>Role</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>histamine (H1), CysLTs</td>
<td>sneezing, nasal congestion</td>
<td>Mizutani et al., 2003</td>
</tr>
<tr>
<td>Tachykinin</td>
<td>Sneezing</td>
<td>Kaise et al., 2001a</td>
</tr>
<tr>
<td>histamine (H1), pLTs</td>
<td>sneezing, nose rubbing, vascular permeability, nasal congestion</td>
<td>Kaise et al., 1998</td>
</tr>
<tr>
<td>platelet activating factor</td>
<td>nasal congestion</td>
<td>Albert et al., 1998</td>
</tr>
<tr>
<td>histamine (H1)</td>
<td>nasal congestion, AHR</td>
<td>Mizutani et al., 1999</td>
</tr>
<tr>
<td>histamine</td>
<td>vascular permeability</td>
<td>Mizuno et al., 1991</td>
</tr>
<tr>
<td>TXA₂, histamine, neuropeptides</td>
<td>nasal congestion</td>
<td>Kaise et al., 2001b</td>
</tr>
<tr>
<td>TXA₂, histamine</td>
<td>vascular permeability, nasal congestion</td>
<td>Yamasaki et al., 1997</td>
</tr>
<tr>
<td>Leukotrienes (B₄, C₄)</td>
<td>exudation, nasal congestion</td>
<td>Shizawa et al., 1997</td>
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<tr>
<td>histamine (H1)</td>
<td>Sneezing</td>
<td>Nabe et al., 2001</td>
</tr>
<tr>
<td>histamine (H1)</td>
<td>nasal congestion</td>
<td>McLeod et al., 2002</td>
</tr>
<tr>
<td>CysLTs</td>
<td>nasal congestion</td>
<td>Fujita et al., 1999</td>
</tr>
<tr>
<td>eosinophils, EPO</td>
<td>nasal congestion, edema, epithelial disruption</td>
<td>Imai et al., 2000</td>
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</tr>
<tr>
<td>eosinophils, TXB₂, pLTs, eNOS, histamine (H₁)</td>
<td>sneezing, nose rubbing, rhinorrhea, nasal congestion</td>
<td>Zhao et al., 2005</td>
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<td>thromboxane, histamine</td>
<td>nasal congestion</td>
<td>Sakairi et al., 2005</td>
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<td>leukotriene D₄, nitric oxide</td>
<td>nasal congestion</td>
<td>Mizutani et al., 2001</td>
</tr>
<tr>
<td>histamine and nitric oxide</td>
<td>nasal congestion</td>
<td>Bockman et al., 2002</td>
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<td>TXA₂, pLTs</td>
<td>nasal congestion</td>
<td>Yamasaki et al., 2001</td>
</tr>
<tr>
<td>constitutively produced nitric oxide</td>
<td>nasal congestion</td>
<td>Imai et al., 2001</td>
</tr>
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**Table 1.3: Inflammatory cells and mediators in rat allergic rhinitis**

<table>
<thead>
<tr>
<th>Mediators and cells</th>
<th>Role and effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines</td>
<td>Sneezing, nose rubbing</td>
<td>Sugimoto et al., 2000a</td>
</tr>
<tr>
<td>histamine (H₁)</td>
<td>Sneezing, nose rubbing</td>
<td>Sugimoto et al., 2000b</td>
</tr>
<tr>
<td>CysLTs</td>
<td>mucus production</td>
<td>Shimizu et al., 2000</td>
</tr>
<tr>
<td>PAF, histamine, serotonin, LTs</td>
<td>Vascular permeability</td>
<td>Albert et al., 1998</td>
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**Table 1.4: Inflammatory cells and mediators in mouse allergic rhinitis**

<table>
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<th>Mediators and cells</th>
<th>Role and effect</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>cytokines (IFN-gamma, IL-2, IL-4)</td>
<td>Sneezing, vascular permeability</td>
<td>Murasugi et al., 2005</td>
</tr>
</tbody>
</table>
expression of iNOS in nasal mucosa | Oh et al., 2003
---|---
TNF-α | Sneezing, nose rubbing, increase expression of adhesion molecules, eosinophils infiltration | Iwasaki et al., 2003
TXA₂, leukotrienes | eosinophil infiltration | Kayasuga et al., 2003
IL-5 | Eosinophilopoiesis | Saito et al., 2002
basophils, eosinophils, CD4+ cells, IL4 and IL5 cells | Sneezing, nose rubbing, AHR | Saito et al., 2001

Table 1.5: Inflammatory cells and mediators in dog allergic rhinitis

<table>
<thead>
<tr>
<th>Mediators and cells</th>
<th>Role and effect</th>
<th>Reference</th>
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<tbody>
<tr>
<td>leukotriene B4</td>
<td>neutrophilia, nasal secretion</td>
<td>Cardell et al., 2000</td>
</tr>
<tr>
<td>histamine</td>
<td>nasal congestion</td>
<td>Tiniakov et al., 2003</td>
</tr>
<tr>
<td>neuropeptide Y</td>
<td>nasal secretion, vasodilatation</td>
<td>Revington et al., 1997</td>
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Table 1.6: Inflammatory cells and mediators in pig allergic rhinitis

<table>
<thead>
<tr>
<th>Mediators and cells</th>
<th>Role and effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>calcitonin gene related peptide</td>
<td>mediate bradykinin and histamine induced nasal congestion</td>
<td>Malis et al., 2001</td>
</tr>
<tr>
<td>histamine (H1)</td>
<td>watery secretion</td>
<td>Szelenyi et al., 2000</td>
</tr>
</tbody>
</table>

1.2.7 Pathophysiological events in allergic rhinitis

1.2.7.1 Neuronal events

Apart from sympathetic and parasympathetic nerves which contain norepinephrine and acetylcholine, respectively, a major role has been attributed to the
sensory nasal innervation (Baraniuk et al., 1991). The participation of sensory airway nerves has been demonstrated to play a key role in allergic rhinitis (Heppt et al., 2004). Nasal sensory nerve fibres contain a number of different peptides, including calcitonin gene-related peptide (CGRP) and the tachykinins, substance P and neurokinin A (NK-A). These neuropeptides, metabolised by the enzyme neutral endopeptidase (NEP), are released from the sensory nerves that form part of the non-adrenergic non-cholinergic (NANC) nervous system, and are capable of generating local reflexes which causes an increase in vascular permeability, plasma leakage, vasodilation and subsequent tissue oedema (Baraniuk 1997). This response is known as neurogenic inflammation, and is mediated by the tachykinin NK-1 and NK-2 receptors. In addition, eosinophils are capable of producing vasoactive intestinal peptide (VIP) and substance P (Metwali et al., 1994). Increased levels of substance P and vasoactive intestinal polypeptide (VIP) in nasal secretions of patients with allergic rhinitis were demonstrated after nasal provocation (Mosimann et al., 1993). Together with other mediators such as neuropeptide Y (Groneberg et al., 2004), calcitonin gene-related peptide (GCRP) (Springer et al., 2003), they may participate in pathophysiological mechanisms underlying allergic rhinitis.

Furthermore, the inflammatory mediators released during the allergic response are able to sensitize and activate the sensory nerve endings by inhibiting neuronal after-hyperpolarization and increasing PKC phosphorylation of neuronal ion channels respectively. Additionally, exposure of nerve ending to cytotoxic proteins (e.g. major basic protein and eosinophil cationic protein) and increases in the expression of receptors
on the neuronal membranes by cytokines (e.g. IL-1β and TNFα) may increase neuronal hyperexcitability (Christiansen et al., 2002).

Neurotrophins change the phenotype of sensory and other types of nerves. Nerve growth factor (NGF) is a potent trophic substance for nerves that can change their phenotype. In sensory nerves, especially C fibers, NGF appears to be the only active neurotrophin. It can be released by several types of cells, including possibly mast cells. NGF can have acute effects that change neuroterminal function. NGF can also reach the nucleus through retrograde transportation up the body of the nerve, producing signals that increase neuropeptide content in these nerves, and stimulate nerve growth. Evidence now exists that NGF is present in the nasal fluids of individuals with active chronic allergic rhinitis. Furthermore, NGF is acutely released upon nasal allergen challenge (Togias 2000; Sanico et al., 2000).

In allergic rhinitis, the most significant effects of sensory neuron activation are itch and the recruited reflexes such as sneeze, the allergic “salute” and bilateral parasympathetic reflexes (Baraniuk et al., 2000; Casale et al., 2002). Upon being activated by different mediators, with histamine being the most prominent, the sensory neurons are depolarized and the depolarization propagates up neurons. In the central nervous system, trigeminal nociceptive neurons enter the pons through the sensory root, and turn caudally in the trigeminal spinal tract to terminate in the pars caudalis of the nucleus of the spinal tract in the lower medulla and upper three cervical segments of the spinal cord. Pars caudalis interneurons cross the midline to enter the trigeminothalamic tract and terminate in the medial part of the ventral posterior thalamic nucleus (arcuate or
semilunar nucleus). Pain and itch stimuli are received at the thalamic level. Connections between the afferent interneurons of the nuclei of the trigeminal spinal tract and the solitary tract with the nucleus ambiguus establish the sneezing reflex. Similar connections regulate parasympathetically mediated glandular secretion in the nose (superior salivatory nucleus and facial nerve) (Calliet 1992).

1.2.7.2 Vascular events

Acetylcholine, catecholamines, various peptides and also nitric oxide participate in nasal vascular control (vasoconstriction or vasodilatation) (Lund 1996). These bioactive molecules arise from both sensory and autonomic nerve fibers, and from neuroendocrine cells widely dispersed in the nasal mucosa. The adult human nasal mucosa exhibits dense nerve networks containing vasoactive intestinal peptide, neuropeptide Y, or its C-terminal peptide, substance P, calcitonin gene-related peptide (CGRP) among others. Sympathetic fibers carry both norepinephrine and neuropeptide Y. Immunoreactivities for neuropeptide Y and C-terminal peptide show full colocalization and are mainly found in perivascular fibers. The subepithelial region contains a dense plexus of substance P and CGRP-immunoreactive fibers, but these nerves also appear around blood vessels (Anggard et al., 1983; Lacroix et al., 1992; Hauser-Kronberger et al., 1993).

Nasal congestion is a common symptom of acute and chronic rhinitis. It is caused by swelling of nasal blood vessels that expand so as to restrict or obstruct airflow through nasal passages (Broms 1982). During allergic reactions, a large number of inflammatory and immunological mediators derived from leukocytes, plasma, and neurons (e.g. leukotrienes, kinins, histamine, neuropeptides, NO, ACh) effect the nasal vasculature by acting on receptors found on different components of blood vessels to cause either
vasodilatation or vasoconstriction (Lung et al., 1984; Widdicombe 1986; Widdcombe 1990). In the inferior turbinate of human nasal mucosa, arterioles and venous sinus are constricted by norepinephrine, neuropeptide Y and endothelin-1 resulting in a sense of decongestion, and dilated by acetylcholine, vasoactive intestinal peptide, nitric oxide, calcitonon gene related peptide and substance P resulting in a sense of congestion (Riederer et al., 2000). Changes in vascular innervation could be one of the factors involved in the maintenance of rhinitis. Nasal vascular hyperinnervation has been detected in patients with allergic rhinitis, as compared with non-allergic individuals (Figueroa et al., 1998).

Plasma extravasation (vascular permeability or exudation) is unfiltered plasma containing albumin, antibodies and complement fractions (Bousquet et al., 1996). An increase in vascular permeability occurs in both naturally occurring seasonal allergic rhinitis, and in perennial allergic rhinitis (Wilson et al., 1998). Additionally, it has been shown that vascular permeability increases after histamine and bradykinin challenge in individuals with allergic rhinitis (Rajakulasingam et al., 1993). In addition, these two vasoactive substances have been shown to increase after allergen challenge (Baroody et al., 1994; Paul et al., 1994). Furthermore, histamine produces concentration-dependent nasal airway exudation of bulk plasma in subjects with seasonal rhinitis (Svensson et al., 1995). The role of kinins in induction of vascular permeability has also been proposed as nasal stimulation with histamine or LTC4 results in an increase of nasal vascular permeability and of kinins concentration in the nasal lavage fluid in allergic rhinitis (Shirasaki et al., 1989).
The increase in vascular permeability is particularly marked in postcapillary venules where the opening of the intercellular gaps together with anatomical fenestrations provides the plasma with an alternative route other than through blood vessels (Widdicombe 1997). The inflammatory mediators act on specific receptors on the blood vessels to cause vascular extravasation through either vasodilation, which causes increase in intravascular pressure especially post capillary venules, or increase interendothelial gaps, and eventually escape of the exudate to the interstitial space and then to the nasal cavity.

Beside nasal congestion and exudation, cellular infiltration also occurs in allergic rhinitis. The expression of adhesion molecules on the endothelial cells induced by acute and chronic released inflammatory mediators enhances the extravasation of different leukocytes to the site of inflammation. The role of the inflammatory cells in allergic rhinitis is described in inflammatory cells section above.

1.2.7.3 Glandular events

Rhinorrhea (watery secretion) is one of the symptoms of allergic rhinitis. Secretions from the nose come from three main sources: the epithelial goblet and serious cells, the submucosal seromucous glands, and the anterolateral deep glands in the nose (Widdicombe et al., 1982; Wells et al., 1986). In addition transudation may contaminate secreted mucus. Nasal mucus secretion is controlled predominantly by parasympathetic cholinergic nerves (Widdicombe 1990). In allergic rhinitis, neurotransmitters (ACh, Substance P) and inflammatory mediators (histamine, bradykinin, leukotrienes) cause increases in glandular secretions (Widdicombe et al., 1982; Wells 1986; Knowles et al.,
1987). In the nasal mucosa of human inferior turbinates, nerve fibers are found in the periglandular tissue around the acini, ducts and in the periglandular connective tissue. It has been found that VIP is in contact with acinus cells and CGRP is found in the connective tissue around glandular cells suggesting a role in controlling glandular secretions (Knipping et al., 2001).

1.2.8 Nasal Airway Hyperresponsiveness (AHR)

Nasal hyperresponsiveness is a hallmark of allergic rhinitis (Druce et al., 1985; Mullins et al., 1989). Subjects with allergic rhinitis show an increased response to nasal challenge with a variety of stimuli, including histamine, bradykinin (both of which are released following allergen challenge), methacholine, tobacco smoke and perfume (Baraniuk 1997; Van Wijk et al., 1999). AHR is associated with nasal congestion, and increased mucus production and oedema following allergen challenge, in both the upper and lower airways. It is usually associated with the late phase reaction, but can continue well beyond this stage. In fact, it is induced irrespective of whether the late phase of inflammation occurs (Togias et al., 1988). Most patients with allergic rhinitis, besides having chronic inflammation in their nasal mucosa that results from allergic reactions, also have chronic inflammation in the lower respiratory tract that can lead to AHR (Ma et al., 2000).

There are a number of potential mechanisms by which AHR might occur: greater receptor activation due to increased mediators release after initial exposure to allergen; increased exposure of receptors to any stimulus present (due to damage and destruction of epithelial and interstitial cells, and mucociliary clearance system by platelet activating...
factor and cytotoxic proteins like major basic protein and eosinophil chemotactic protein); reduced the metabolism of the mediators (due to loss of epithelial function); increased receptor expression (e.g. methacholine causes more secretion in allergic subjects than in non-allergic subjects); and alteration of intracellular pathways (Laitinen et al., 1985; Devillier et al., 1988; Koga et al., 1992; White 1993; Teixeira et al., 1997).

1.2.9 Overall summary

In this introduction, the human nose was discussed in detail. Since most pre clinical studies in determining human nasal physiology, histopathology and pathogenesis of allergic diseases are conducted using animal tissues, comparisons between human and experimental animals was discussed in detail to assess similarities and differences in terms of structure and function of the nose, and to evaluate the value of such studies in applying and generalizing them to humans. There was extensive discussion of the guinea pig since this thesis deals with guinea pig allergic rhinitis. There are similarities and differences in terms of distribution and function of different component of the nose (histology, innervation, and vasculature). The pathogenesis of allergic rhinitis is complex. It involves neuronal, vascular, and glandular events. Mediators eliciting allergic responses which are released by inflammatory cells (e.g. mast cells, eosinophils, lymphocytes, basophils and epithelial cells) act in a synergistic way to produce allergic inflammation. They include cytokines, chemokines, autacoids, neurotransmitters, neuropeptides and kinins.
1.3 Drug targets in allergic rhinitis

1.3.1 Current registered drugs: limitations to their use

The two major classes of drugs used to treat symptoms of allergic rhinitis are oral H1 antihistamines and intranasal corticosteroids. These agents may be used as monotherapy or in combination, depending on the predominant symptoms and the patient’s response to therapy. Alternative agents, such as cromolyn sodium, may be appropriate in some patients. The first-generation antihistamines include brompheniramine, chlorpheniramine, and diphenhydramine. Although these drugs relieve the sneezing and rhinorrhea in allergic rhinitis, they cross the blood–brain barrier and are associated with marked drowsiness and impaired mental performance. The second-generation antihistamines produce no, or considerably less, sedation than the first-generation drugs. However, oral terfenadine and astemizole produced potentially dangerous (fatal) cardiac arrhythmias in some patients (Day, 1999) and have been removed from the market.

The newer second-generation antihistamines (acrivastine, cetirizine, fexofenadine, desloratadine, loratadine) are not so associated with such troubling side effects (Kay, 2000). Fexofenadine, loratadine, and desloratadine are considered nonsedating antihistamines. Acrivastine may be sedating in some patients. Cetirizine is considered low sedating. Oral antihistamines are also first-line therapy for allergic rhinitis in children (Dykewicz et al., 1998). These drugs have demonstrated efficacy in the relief of the seasonal and perennial rhinitis symptoms of sneezing, itching, and nasal discharge. They have also been found to reduce ocular symptoms of allergic conjunctivitis, which frequently occur in conjunction with allergic rhinitis.
H1 antihistamines are generally not considered effective for nasal congestion (Dykewicz et al., 1998). Therefore, in patients with this symptom, combination therapy with an oral antihistamine plus a decongestant may be helpful. Such combinations are available in convenient fixed-dose products that can be taken once daily. However, such decongestants as pseudoephedrine and phenylpropanolamine can have unwanted effects, such as insomnia, loss or stimulation of appetite, and should be used with caution in patients with conditions such as arrhythmias or angina.

Intranasal H1 antihistamines, such as azelastine and levocabastine, are also first-line therapy for mild-to-moderate allergic rhinitis (Dykewicz et al., 1998). These topical antihistamines are administered twice daily, and have a rapid onset of action. Both azelastine and levocabastine have been shown to improve symptoms in patients with seasonal or perennial allergic rhinitis (Bousquet et al., 2001), and they appear to have the potential to reduce nasal congestion.

Intranasal corticosteroids are considered first-line treatment for more severe symptoms of allergic rhinitis (Dykewicz et al., 1998; Bousquet et al., 2001). The most effective medications for controlling allergic rhinitis are nasally inhaled corticosteroids including beclomethasone, budesonide, flunisolide, mometasone, and triamcinolone (Corren 2000; Kaszuba et al., 2001). Corticosteroids target the inflammatory mechanisms. Thus, intranasal steroids are particularly effective in ameliorating nasal congestion, which is often the main complaint in chronic allergic rhinitis, but they also relieve the other symptoms of rhinitis, such as rhinorrhea, sneezing, and nasal itching. Most are administered once or twice daily. Intranasal corticosteroids should be administered continuously. For optimal benefit therapy should begin before the onset of
symptoms (for example, before pollen season). The onset of action is slower with intranasal corticosteroids than with oral antihistamines. Maximum benefit usually occurs over days or weeks. Systemic side effects appear to be minimal in adults receiving intranasal corticosteroids. Nose bleeding or irritation may occur with the use of intranasal corticosteroids although these effects may diminish over time. In rare cases, septal perforations may develop.

Intranasal cromolyn sodium has been shown to relieve allergic rhinitis symptoms in some patients. It needs to be initiated before the onset of symptoms and does not improve symptoms once they occur. It may need to be administered up to four times daily.

Immunotherapy may be appropriate in patients who have severe symptoms whose symptoms are caused by allergens for which potent extracts are available, and who have not responded to pharmacotherapy (Dykewicz et al., 1998). Age and concomitant illnesses are among the factors that determine whether immunotherapy is appropriate. For example, immunotherapy is rarely appropriate in preschool children, the elderly and in those with severe pulmonary or cardiovascular disease. In general, effective immunotherapy requires three to five years of treatment.

Due to redundancy, synergy and pleiotropism existing amongst the mediators of allergic rhinitis, there are limitations to the currently available drugs in terms of their effectiveness. Antihistamines (H1) cause symptomatic relief only, and steroids do not provide acute relief of symptoms and they are nonselective, unsuitable for some patients (especially in pediatric use), may cause nasal irritation, bleeding and in rare cases even systemic side effects. In addition, timing of treatment is critical with anti IgE and
immunotherapy needs identification of allergen and multiple injections. Moreover, anticholinergics are useful only in reducing rhinorrhea.

1.3.2 Possible novel targets for treating allergic rhinitis

An improved understanding of the cellular and molecular mechanisms underlying the pathogenesis of allergic rhinitis has resulted in the identification of potential novel therapeutic strategies. In theory, the inhibition of an upstream pathway in the allergic cascade (for example, dendritic cells or TH2 cells) is likely to make a greater clinical contribution, compared with the inhibition of a single downstream mediator (Holgate et al., 2003).

1.3.2.1 Mediator inhibitors

1.3.2.1.1 Antihistamines

The new generation of H1 antihistamines show greatly improved efficacy and safety because they act as inverse agonists (stabilize the inactive conformation of the receptor and drive the equilibrium away from the active conformation which leads to reduction of the constitutive activity of the receptor and inhibition of basal activity) (Oppenheimer et al., 2002). A number of antihistamines (e.g. fexofenadine) are also claimed to exert anti-inflammatory actions (Baroody et al. 2000). The recent discovery of the H4 receptor expressed on mast cells, basophils and eosinophils has generated renewed interest in histamine because H4 receptors mediate Ca\textsuperscript{2+} signalling and chemotaxis.
(Hofstra et al., 2003). It is possible that selective H₄ antagonists could have anti-inflammatory actions in allergic disease.

1.3.2.1.2 Leukotrienes inhibitors

Cysteinyi leukotrienes (CystLTs) are released from mast cells, basophils, eosinophils and macrophages and are particularly important in causing nasal blockade (Higashi et al., 2003). Clinical trials of montelukast and zafirlukast (CystLT₁ receptor antagonists) in allergic rhinitis have demonstrated that their effectiveness is overall less than that of topical nasal corticosteroids (Pullerits et al., 2002; Philip et al., 2002; Topuz et al., 2003). The recent discovery of both CystLT₁ and LT₂ receptors on eosinophils, which differ in their avidity for the leukotrienes LTD₄ and LTC₄, indicates a potential pro-inflammatory role for this mediator class that could be usefully inhibited by a dual antagonist (Evans 2002).

1.3.2.1.3 Prostaglandin receptor antagonists

Among the prostaglandins, PGD₂, which is a mast-cell-derived eicosanoid, has potent vasodilator properties mediated by the DP₁ receptor. DP₁ receptor antagonists offer promise for situations in which nasal blockade is problematic. A second PGD₂ receptor, DP₂ has been identified as a T₄₂ marker but is also expressed on eosinophils and basophils where it serves a chemotactic function antagonism of which could have anti-inflammatory effects (Arimura et al., 2001; Holgate et al., 2003; Sugimoto et al., 2003).
1.3.2.1.4 Tryptase antagonists

Mast-cell granules contain high concentrations of protease, tryptase. Tryptase exerts a range of inflammatory responses that have been implicated in chronic tissue injury and remodelling possibly involving coagulation Factor II receptor-like 1 found on epithelial cells, fibroblasts and smooth muscle. A number of tryptase inhibitors have been described and some efficacy in humans has been reported in allergic models, including allergen challenge (Newhouse 2002).

1.3.2.1.5 Nitric oxide synthase (NOS) inhibitors

There is an increase in level of nitric oxide production in allergic rhinitis. Nitric oxide causes vasodilation and glandular secretion (Baraniuk 1997). NOS inhibitors have been shown to reduce nasal blockade in perennial allergic rhinitis and plasma extravasation in seasonal allergic rhinitis (Dear et al., 1996). However, NOS inhibitors, when nasally administered to human subjects, caused the development of upper airway hyperreactivity and significant eosinophilia (Turner et al., 2000).

1.3.2.2 Mast cell stabilizers

1.3.2.2.1 Cromones

Sodium cromoglycate, and its successor nedocromil sodium, are thought to act as mast cell stabilizers, but their precise mechanism(s) of action is not known. They have been shown to be effective in reducing immediate phase symptoms in allergic rhinitis (Kunkel et al., 1987).
1.3.2.2 Protein kinase inhibitors

Activation of Syk kinase, a transducer of signaling through the Fcε receptor of mast cells via the binding of ligand to IgE bound to the IgE receptor leads to an array of responses including degranulation and neosynthesis of proinflammatory mediators. In a clinical study, inhibitor of Syk kinase (R112) has been shown to be effective in relieving the symptoms of allergic rhinitis (Meltzer et al., 2005). Moreover, The finding that genistein, a potent inhibitor of tyrosine kinase, has potent anti-inflammatory activity on the mast-cell-dependent early- and late-phase allergen-provoked inflammatory reaction in the airways of guinea pigs provides proof of concept for selective inhibitors of protein kinases linked to mast-cell activation (Duan et al., 2003).

In addition, mast cells also express receptors that are able to inhibit IgE-dependent degranulation through the activation of immuno-receptor tyrosine-based inhibitory motifs (ITIMS). On associating with the FcεR1, inhibitory receptors, such as immunoglobulin-like transcripts (ILTs) and leukocyte immunoglobulin-like receptors (LIRs), are able to affect IgE signalling by triggering phosphorylation of ITIM sequences on the gamma-chains of FcεR1. At present, 13 LIRs are recognized, LIRs 1, 2, 3, 5 and 8 have inhibitory effects. LIR5 (gp49A and gp49B) is expressed at a high level by mast cells (Katz et al., 1996). Although no natural ligands for these receptors have yet been identified, they offer targets at which to direct novel inhibitory agents.

In the process of their differentiation, survival and optimal secretion, mast cells are dependent on stem-cell factor (SCF) (Costa et al., 1996). In disorders such as mastocytosis, blockade of SCF Src kinase activity by the selective inhibitor PP1 has a marked effect in suppressing mast-cell proliferation (Tatton et al., 2003). Ablation of
mast cells in the nasal mucosa would clearly have a large benefit in allergic rhinitis, where the mucosal mast-cell population markedly increases.

1.3.2.2.3 Ion channels blocking drugs

Selective blockers of inwardly rectifying and Ca$^{2+}$-activated K$^{+}$ channels, and Ca$^{2+}$-independent Cl$^{-}$ channels linked to IgE-dependent activation, expressed in mast cells may also offer promise for a new generation of anti-allergic drugs (Duffy et al., 2001).

1.3.2.3 Inhibitors of neuronal pathways

Allergic rhinitis is characterized by local neural activity, such as itching, sneezing and reflex-mediated secretion (Baraniuk 1992; Barnes 2001). Although substance P induces eosinophilia in allergic rhinitis (Fajac et al, 1995), inhibition of its receptors (neurokinin 1 and 2) has so far proven disappointing when tested in clinical trials. For bradykinin, which is a potent releaser of neuropeptides, efficacy of a bradykinin B$_{2}$ agonist has been reported in nasal allergen challenge (Austen et al., 1994), but subsequent Phase III clinical trials proved disappointing. Other neuropeptides also provide interesting targets, including calcitonin-gene-related peptide (CGRP) in chronic vasodilation (Uddman et al., 1999) and secretoneurin, which is present in cholinergic, adrenergic and sensory nerves (Korsgren et al., 2003), and which exerts a pro-inflammatory effect on eosinophils (Dunzendorfer et al., 1998). If suitable antagonists for these mediators are found then they are likely to be efficacious in the more chronic forms of allergic rhinitis in which nasal blockade dominates.
1.3.2.4 Immunotherapy

Certain strategies have been used in immunotherapy. These include the use of allergen-specific immunotherapy, allergen peptide-based immunotherapy, and DNA immunotherapy.

1.3.2.4.1 Allergen-specific immunotherapy

The goal of allergen-specific immunotherapy (ASIT) is to modulate the immune response to allergen and thereby reduce the symptoms of allergic rhinitis. ASIT is administered as a series of subcutaneous injections, or sublingually, of highly purified airborne allergen(s) with a dose of 6-24 μg to patients with allergic rhinitis who are specifically sensitized to identified allergen(s). ASIT is clinically effective in reducing symptoms of allergic rhinitis, as evidenced by the inhibition of both the allergen-provoked early- and late-phase nasal responses and, in children sensitized to a single allergen, a reduced risk of the subsequent development of sensitization to further allergens (Pajno et al., 2001; Malling 2002; Moller et al., 2003). The mechanisms through which ASIT produces its beneficial clinical effects are becoming clear (Canonica et al., 2003). ASIT reduces clinical symptoms by inhibiting allergen-specific T_{H2} cells in favour of a T_{H1} response (immune deviation) (Walker et al., 2002), and inducing regulatory lymphocytes carrying the CD4 and CD25 antigens and CD4+ CD25- T_{H3} (immune tolerance) (McHugh et al., 2002). Specific immunotherapy also increases allergen-specific 'blocking' IgG1 and IgG4 antibodies, a variable decline in allergen-specific IgE, and reduces both the number and activation state of mucosal mast cells, basophils and eosinophils (Ebner 1999). Even though SIT is efficacious, its
administration can be associated with local and systemic allergic reactions, and so a variety of strategies to reduce this, and to enhance efficacy, are being investigated.

1.3.2.4.2 Peptide-based immunotherapy

The rationale for using short peptides is to reduce the potential for allergic side effects while retaining the beneficial effect of peptide epitopes recognized by T cells in modifying their response to allergens, because peptides are unable to crosslink FcεR1-bound IgE on mast cells and basophils (Holgate et al., 2003). The safety and efficacy of peptides has been taken advantage of in the treatment of cat allergy using several overlapping peptides derived from chain 1 or 2 of the major cat allergen Fel d1. Weekly subcutaneous immunization with 27-amino-acid peptides derived from Fel d1 led to a reduction in rhinitis symptoms on exposure to cats (Norman et al., 1996).

1.3.2.4.3 DNA immunotherapy

Immunostimulatory DNA sequences containing CpG motifs are strong inducers of a T_{H1} immune response to antigen, and have therefore been investigated in the treatment of T_{H2}-mediated diseases such as allergic rhinitis and asthma (Horner et al., 2001a). CpG DNA inhibits T_{H2} responses to antigen indirectly by influencing the function of cells of the innate immune system, rather than exerting direct effects on T lymphocytes. Studies with TLR9-deficient mice have demonstrated that these receptors of the innate immune response are essential in mediating the immunostimulatory activity of CpG DNA, which is characterized by the production of IL-12, IL-18, interferon
gamma, IL-6 and IL-10 (Roman et al., 1997; Hemmi et al., 2000). The cytokine environment induced by CpG DNA is highly effective at reducing the levels of expression of Th2 cytokine receptors (for example, the IL-4 receptor) (Horner et al., 2001b). In a mouse model of allergic rhinitis, CpG DNA administration prevented both the development of nasal symptoms and eosinophilic inflammation (Hussain et al., 2002).

1.3.2.5 IgE targeting

To avoid sensitization with foreign proteins, a humanized monoclonal antibody containing 95% human IgG1 and 5% murine IgE-binding epitope has been constructed (Presta et al., 1993). This antibody recognizes IgE selectively, inhibits binding of IgE to both FcεRI and FcεR2, and therefore fails to initiate mast-cell or basophil activation. By this mechanism, omalizumab therapy is accompanied by a marked reduction in inflammatory leukocytes and expression of FcεRI, which, if not occupied by IgE, becomes internalized (Plewako et al., 2002). When administered as two-weekly, or one-monthly subcutaneous injections, omalizumab decreases circulating free IgE by >90% by forming small (1000 kDa), non-complement-fixing complexes that are eliminated by the reticuloendothelial system without causing side effects. In clinical trials of seasonal allergic rhinitis, omalizumab has shown efficacy (Adelroth et al., 2000; Casale et al., 2001). Furthermore, in children with allergic rhinitis, a combination of SIT with anti-IgE for 24 weeks was more efficacious than when either treatment was given alone (Kuehr et al., 2002).
1.3.2.6 Cytokines and chemokines inhibitors

One of the difficulties in deciding which cytokines or chemokines to target the treatment of allergic rhinitis is the large variety of these that are expressed at sites of allergic inflammation, as well as their overlapping functions. In allergic inflammation, research has focused particularly on individual Th2 cytokines (for example, IL-4, IL-5, IL-9 and IL-13) and chemokines that attract cells to sites of allergic inflammation (Kay 2001).

To date, there are no published studies of cytokines antagonists in humans with allergic rhinitis. However, animal studies and cytokine challenges in humans help illustrate their effect in nasal allergy. The therapeutic potential of a recombinant soluble IL-4 receptor (Nuvance) as an IL-4 antagonist has shown improvement in asthmatics (Borish et al., 2001). In addition, monoclonal anti-IL-4 antibodies inhibit IgE production in mice (Zhou et al., 1997). Allergen challenge increases the level of expression of IL-13 in the nasal mucosa in vivo, whereas, in vitro, IL-13 increases the number of secretory cells in human nasal epithelial cells (Wills-Karp et al., 1998; Skowron et al., 2003). Targeting IL-13 has been investigated in allergic inflammation in mouse models of asthma, and its antagonism inhibits the allergic inflammatory response in the lower airways (Wynn et al., 2003).

The C-C chemokines, including eotaxin, RANTES, monocyte chemoattractant proteins 1 and 3, are particularly relevant to allergic inflammation, since increased levels of these chemokines are detected in the nasal mucosa following allergen challenge and all interact with the CCR3 receptor on eosinophils, basophils and mast cells (Terada et al., 2001). Activation of CCR3 receptors by application of eotaxin to the nasal mucosa
induces an influx of eosinophils (Gorski et al., 2002). Studies demonstrating that an 11-
amino-acid synthetic peptide inhibits nasal influx of neutrophils and protein exudation
induced by nasal challenge with IL-8 in normal subjects indicate the potential for
inhibiting the function of chemokines in the nasal mucosa (Cooper et al., 2001). Given
the importance of the issue, three chemokine receptors CCR3, CCR4 and CCR8
preferentially expressed by Th2 cells, mast cells or eosinophils therefore represent
therapeutic targets in allergy.

1.3.2.7 Adhesion molecules inhibitors

Adhesion molecules expressed on leukocytes and endothelial cells are important
for inflammatory cell recruitment during allergic inflammation. At present, there are no
published studies of anti-adhesion therapy in allergic rhinitis. However, targeting of these
molecules on leukocyte or endothelial cell surfaces has been investigated as an approach
to inhibiting allergic inflammation. Among these molecules is endothelial P-selectin,
which is highly expressed in the nasal mucosa and has been shown to stimulate
eosinophil recruitment in mouse models of allergic inflammation (Symon et al., 1994).
Subsequent to endothelial tethering, eosinophils firmly adhere to either ICAM-1 or
VCAM-1. Blockade of these receptors in mouse allergic inflammation, and inhibition of
eosinophilic tissue recruitment in ICAM-1-deficient mice, results in marked inhibition of
adhesion of eosinophils to endothelium (Broide et al., 1998). Furthermore, eosinophils,
basophils, monocytes and T cells, but not neutrophils, express high levels of very late
antigen-4 (VLA-4), the ligand for VCAM-1 (Jackson 2002). Binding of VLA-4 to the
CS-1 region of fibronectin also induces eosinophil activation (Anwar et al., 1993), such that by targeting VLA-4, cell activation as well as cell recruitment might be inhibited.

1.3.2.8 Selective phosphodiesterase 4 inhibitors

One promising development is the use of selective phosphodiesterase 4 (PDE4) inhibitors which exert anti-inflammatory activity by blocking the hydrolysis of cyclic 3'5'-AMP in lymphocytes, eosinophils, neutrophils and monocytes, thereby attenuating their release of mediators and cytokines (Giembycz 2000). Although known to be effective in the treatment of asthma and chronic obstructive pulmonary disease, oral once-daily therapy with the PDE4 inhibitor roflumilast in patients with allergic rhinitis subjected to repeated allergen exposure proved to be efficacious, especially on nasal blockade (Sorbera et al., 2000; Schmidt et al., 2001).

1.3.2.9 Heparin

Heparin, a straight-chain, highly sulfated glycosaminoglycan, is present in mast cells at high concentrations. Anti-inflammatory and anti-allergic properties of heparin have been demonstrated in several in vitro and in vivo studies (Matzner et al., 1984; Lider et al., 1990). Despite its effects on asthma, very little research has been undertaken over the past years to position heparin in the treatment of allergic rhinitis. In a clinical study, intranasal heparin significantly reduced symptom scores 10 min after antigen challenge. In addition, eosinophil influx in airway mucosa, and eosinophil cationic protein concentrations in nasal lavage fluids were reduced (Vancheri et al., 2001). Moreover,
heparin, prevents nasal mucosa mast cell degranulation induced by adenosine monophosphate (Zeng et al., 2004).

1.3.2.10 Phototherapy

Ultraviolet (UV) light has been shown to exert both local and systemic immunosuppression (Salo et al., 2000; Duthie et al., 2000), and has been widely used for decades in the therapy of various skin diseases. The major mechanisms for UV irradiation-induced immunosuppression involves induction of apoptosis in infiltrating T cells, reductions in the number of Langerhans cells and their function, and induction of immunosuppressive cytokines such as interleukin-10 in the skin (Garssen et al., 2001; Nghiem et al., 2002). In addition, UV irradiation inhibits histamine release from mast cells in vitro and in vivo (Gollhausen et al., 1985; Danno et al., 1988). Recently, the immunosuppressive action of UV has attracted researchers to apply this technique in allergic rhinitis. In a clinical study, intranasal irradiation with the 308nm xenon chloride (XeCl) ultraviolet-B laser and irradiation with a combination of ultraviolet-B (UVB), ultraviolet-A (UVA) and visible light (VIS) was effective in treating allergic rhinitis (Csoma et al., 2004; Koreck et al., 2005). Furthermore, intranasal therapy with 8-methoxypsoralen (8-MOP) plus UVA light three times weekly for three weeks inhibited significantly the symptoms of allergic rhinitis (sneezing, rhinorrhea, itching and congestion) (Csoma et al., 2006). These results suggest that intranasal phototherapy is effective in the treatment of allergic rhinitis.
1.4 Methods for studying allergic rhinitis in experimental animals

1.4.1 General overview

Rhinitis is induced in guinea pigs, rats, mice, dogs and pigs using different kinds of allergens with various methods of sensitization. The sensitized animals produce the different symptoms and signs of allergic rhinitis including sneezing, nasal itching, nasal congestion and rhinorrhea following allergen provocation. Sneezing and nasal scratching can be assessed immediately after allergen challenge in sensitized guinea pigs, rats, and mice. Nasal secretions can be studied by weighing nasal secretions absorbed onto cotton swabs or filter papers in sensitized guinea pigs, dogs and pigs after allergen challenge. Nasal congestion can be assessed by measuring nasal resistance to air flow using forced air passed to nasal cavities; nasal passage space using acoustic rhinometry; and air flow using plethysmogram in guinea pigs, dogs and pigs. Cellular and biochemical changes can be quantified from nasal lavage collected from sensitized animals.

1.4.2 Techniques used to assess pathophysiological changes in allergic rhinitis in guinea pigs in vivo

After a challenge dose of a specific allergen, sensitized guinea pigs produce the acute and the chronic signs and symptoms of allergic rhinitis including sneezing, nose rubbing, rhinorrhea, and nasal congestion. In addition, there are cellular and biochemical changes. The way these changes are evaluated varies from one study to another. The following is a summary of the different techniques used for evaluation of allergic rhinitis signs and symptoms, and quantification of inflammatory changes in vivo in guinea pigs.
1.4.2.1 Sneezing and Nose rubbing

Sneezing and itching are typical, mostly histamine-mediated signs of allergic rhinitis. Itching cannot be detected directly in guinea pigs. However, it may be related to nose scratching or rubbing combined with sneezing. Conscious guinea pigs are kept individually in cages in a quiet environment and sneezing and nose rubbing are counted by direct observation or recorded using digital camera for later revision. In most cases, sneezing and nose rubbing are counted immediately after allergen provocation and evaluated for a period ranging from first ten minutes to one hour (Kaise et al., 1998; Yamasaki et al., 2001; Nabe et al., 2001; Mizutani et al., 2003; Fukuda et al., 2003; Zhao et al., 2005).

1.4.2.2 Nasal airway pressure

Nasal blockade (decreased nasal patency) can occur as a result of swelling of the nasal mucosa due to vasodilatation of cavernous tissue, and increase glandular secretions. The symptom of congestion in allergic rhinitis is biphasic in time. It occurs during an acute phase as well as a late phase of allergic rhinitis. In sensitized guinea pigs, both phases of nasal congestion have been detected, with the acute phase occurring during the first 30 minutes, and a later phase 4 to 6 hours after exposure to allergen. The degree of patency of the nasal cavity can be measured in both conscious and anaesthetized guinea pigs. In conscious guinea pigs, a two chambered double-flow plethysmograph has been used to measure air flow through the nasal cavity. In this technique, a guinea pig is placed with its neck extending through the partition of a two chambered box (Albert et al., 1998; Mizutani et al., 1999; Fujita et al., 1999; Imai et al., 2000; Nabe et al., 2001;
Yamasaki et al., 2002; Mizutani et al., 2003; Fukuda et al., 2003). According to this technique, nasal air flow is inversely proportional to nasal blockade. Although the use of plethysmograph has gained much attention, its use to measure nasal blockade has been criticized by Swedish researchers who consider that changes in resistance measured in the plethysmograph originate at or below the larynx (Finney et al., 1994). Recently, respiratory rate has been used in conscious guinea pigs to reflect resistance changes in the upper airway (Zhao et al., 2005).

In anaesthetized guinea pigs, nasal blockade can be measured using different methods including a ventilator flow method (Mizuno et al., 1991; Yamasaki et al., 1997; Shizawa et al., 1997; Albert et al., 1998; Fukuda et al., 2003; Sakairi et al., 2005), forced oscillation method (Narita et al., 1998; Mcleod et al., 2002), and acoustic rhinometry (Kaise et al., 1998; Kaise et al., 2001a). In a ventilator flow method, pulsatile air (4-10 ml/stroke and 50-70strokes/minute) is forced toward the nasal cavity from the tracheal side. Any change in air flow resistance is reflected as a nasal patency change. In the forced oscillation method (know also as flow pressure method), one side of nasal cavity is cannulated and the flow of humidified air is restricted from cannulated side through the other side of the nasal cavity and out the nostril. Any change in nasal patency causes reduction in air flow. Acoustic rhinometry is used to measure the volume of the nasal cavity. In guinea pigs, the device can measure changes in the nasal cavity within 2 cm from the nostrils. The volume of the nasal cavity decreases when nasal blockade increases and vice versa.
1.4.2.3 Nasal secretions (Rhinorrhea)

Rhinorrhea is a troublesome symptom of allergic rhinitis. Watery secretions can be produced in sensitized guinea pigs after allergen provocation although in most cases its production is not enough for easy evaluation. The usual quantification of nasal secretions in most studies is gravimetrically. A piece of cotton thread dyed with flourescein can be inserted into the anterior naris of guinea pigs for one minute. The stretch of color is proportional to fluid volume and to increase in weight of a thread due to absorbed nasal secretions (Namimatsu et al., 1991). Alternatively, a pre weighed cottonwool or cotton swab can be used to absorb the secretions from the anterior naris (Fujita et al., 1999; Fukuda et al., 2003). The weight gained is proportional to nasal secretions. In addition, a pre weighed filter paper strip can be continuously inserted into the nares and secretions absorbed for a period of 10 minutes. The increase in paper weight is proportional to nasal secretions (Zhao et al., 2005).

1.4.2.4 Exudation

Exudation occurs as a result of increases in vascular permeability. In sensitized guinea pigs, exudation can be quantified after allergen provocation. Different dyes have been used for this purpose, including Evan’s blue (Mizuno et al., 1990; Mizutani et al., 1999; Mizutani et al., 2001), pontamine sky blue (Yamasaki et al., 1997; Kaise et al., 1998) and brilliant blue (Shizawa et al., 1997). A dye whose, at concentrations wich vary from one study to another (1-10%), is administered intravenously before allergen provocation in anaesthetized guinea pigs. After allergen provocation, nasal cavities are perfused with saline at a rate of 0.2-0.25ml/min for 10 to 20 minutes. Dye concentration
in the collected perfusate is quantified by spectrophotometer using absorbance at 620nm. Dye concentration is proportional to vascular permeability (exudation). Instead of using dye, intravenously administered $^{125}$I-labelled human serum albumin can be quantified after recovery from nasal cavities, and can be used to reflect exudation of serum proteins (Elovsson et al., 2005).

### 1.4.2.5 Cellular and Biochemical changes

Allergic rhinitis basically results in an inflammatory reaction in the nasal mucosa. This can be investigated by measurement of mediators and inflammatory cells in nasal lavages. In sensitized guinea pigs, cells and mediators of inflammation can be detected in nasal lavage in both the acute and chronic phases of allergic rhinitis. Nasal lavage can be collected from anesthetized guinea pigs by perfusion of nasal cavities with saline from the tracheal side (Yamasaki et al., 1997; Shizawa et al., 1997; Imai et al., 2000; Kaise et al., 2001a; Elovsson et al., 2005). Alternatively, saline can be instilled into one nostril and sucked out from the other nostril simultaneously by applying a negative pressure (Mizutani et al., 2001; Yamasaki et al., 2001; Yamasaki et al., 2002; Zhao et al., 2005). Recovered nasal lavage is centrifuged and the resultant supernatant is used for quantitative measurement of mediators (e.g. thromoxanes, leukotrienes, eosinophil peroxidase, NO$_2^-$, NO$_3^-$, histamine) using ELISA or radioimmunoassay. Furthermore, nasal lavage can be assessed for total leukocyte count using hemocytometer or semiautomated haematoloty analyzer, and for differential cell count using cytospin followed by staining and observation under a microscope.
1.5 The rationale and aims of this project

The prevalence of allergic rhinitis is substantial and the financial and social impact of the disease is significant. The effectiveness of currently available drugs for allergic rhinitis is limited. Therefore, the discovery of more effective drugs with fewer side effects is important. In order to evaluate the effectiveness of new drugs, experimental studies in animals with allergic rhinitis are required. Conventionally, guinea pigs have been the species of choice for the evaluation of chemical-related respiratory allergy, primarily because it is possible in this species to elicit and measure with relative ease challenged-induced inflammatory reactions that resemble in some ways the acute clinical manifestations of human allergic rhinitis. Thus, this project was aimed to:

1. establish comprehensive models of allergic rhinitis in guinea pigs

2. investigate possible inflammatory mediators of acute phase reactions of allergic rhinitis in guinea pigs in vivo.

To achieve this, the following experiments were conducted:

1. Systematic evaluation of the models through the assessment of the effect of ovalbumin/saline on acute symptoms of allergic rhinitis including sneezing, nose rubbing, nasal airway pressure and cellular infiltration during the sensitization period and on challenge days.

2. Evaluation of the effect of the autacoid histamine and the neurotransmitter acetylcholine on nasal airway pressure and lung inflation pressure and investigation of the receptors involved in those responses.

3. Investigation of the effect of autacoids (histamine, leukotriene D4 and nitric oxide) on sneezing, nose rubbing, nasal airway pressure and cellular infiltration
during the acute allergic reactions in sensitized ovalbumin challenged guinea pigs using different antagonists.

4. Assessment of the anti-inflammatory activity of heparin and dexamethasone by evaluation of their effect on sneezing, nose rubbing, nasal airway pressure and cellular infiltration in sensitized ovalbumin challenged guinea pigs.
2. Materials and Methods

2.0 Materials

The materials used in the following studies are shown below:

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmacological agents</strong></td>
<td></td>
</tr>
<tr>
<td>4-Diphenylacetaxy-N-methylpiperidine methiodide (4-DAMP)</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>Fluka, Switzerland</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Heparin</td>
<td>Fisher Scientific, Canada</td>
</tr>
<tr>
<td>L-NAME</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>Sigma, U.S.A</td>
</tr>
<tr>
<td>Methoctramine</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Montelukast</td>
<td>Merck, Canada</td>
</tr>
<tr>
<td>Ovalbumin Grade V</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Pentobarbital sodium</td>
<td>Bimeda-MTC, Canada</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td><strong>Cell staining dyes</strong></td>
<td></td>
</tr>
<tr>
<td>Methylene blue</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Wright stain</td>
<td>Fluka, Germany</td>
</tr>
<tr>
<td><strong>Solvents</strong></td>
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</tr>
<tr>
<td>Dextros</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Dimethylsulfoxide (DMSO)</td>
<td>Sigma, Germany</td>
</tr>
<tr>
<td>Polyethylene glycol (PEG)</td>
<td>Fisher Scientific, U.S.A</td>
</tr>
<tr>
<td>Lactose</td>
<td>Pharmacy dept. UBC, Vancouver, Canada</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Sigma, Germany</td>
</tr>
</tbody>
</table>
### Equipment

<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minute epoxy syringe Glue</td>
<td>Henkel, Canada</td>
</tr>
<tr>
<td>Balance (mettler PC 4400)</td>
<td>Mettler instrument, Switzerland</td>
</tr>
<tr>
<td>Electron balance (mettler AE260)</td>
<td>Mettler instrument, Switzerland</td>
</tr>
<tr>
<td>Filter cards thin brown</td>
<td>Thermo-Electron Corporation, UK</td>
</tr>
<tr>
<td>Grass model 79D</td>
<td>Grass instrument Co. Quincy, mass, U.S.A</td>
</tr>
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<td>Hemocytometer</td>
<td>Bright Live, Reichert, U.S.A</td>
</tr>
<tr>
<td>IV Catheter 14G</td>
<td>Medex, U.S.A</td>
</tr>
<tr>
<td>Light microscope</td>
<td>Carsen, Canada</td>
</tr>
<tr>
<td>Micropipette</td>
<td>Gilson, France</td>
</tr>
<tr>
<td>Micropipette tips (1-200ul)</td>
<td>TipOne, Germany</td>
</tr>
<tr>
<td>Microscope cover glass</td>
<td>Fisher Scientific, Canada</td>
</tr>
<tr>
<td>Microscope slides</td>
<td>VWR international, Canada</td>
</tr>
<tr>
<td>Needle 23 G1</td>
<td>Becton Dickinson, U.S.A</td>
</tr>
<tr>
<td>Polyethylene tubing (PE50)</td>
<td>Becton Dickinson, U.S.A</td>
</tr>
<tr>
<td>Pressure transducer</td>
<td>Astromed, Canada</td>
</tr>
<tr>
<td>Shandon Cytospin1</td>
<td>Thermo Electron Corporation, U.S.A</td>
</tr>
<tr>
<td>ULTRA-NEB 99</td>
<td>The DeVILBISS Co. Canada</td>
</tr>
<tr>
<td>Ventilation pump</td>
<td>Harvard apparatus Limited</td>
</tr>
</tbody>
</table>

### 2.1 Methods

#### 2.1.1 Methods of sensitization used in the study

The sensitization procedure used was first described by Yamasaki 1997. Guinea pigs were initially exposed to 1% ovalbumin in saline as a 1% aerosol twice for ten minutes, 7 days apart. The aerosol was generated by an ultrasonic nebulizer (ULTRA-
NEB 99) and a ventilation pump set at 4ml/stroke, 70 strokes/min. On days 14, 15 and 16, a booster of 1% ovalbumin in saline was instilled intranasally at a volume of 20μl/nostril/day into both nostrils. The instillation was performed using a micropipette (200μl capacity). On day 21 guinea pigs were challenged with 2% ovalbumin in saline instilled intranasally at a volume of 20μl/nostril to both nostrils. The sensitization process is shown in Figure 2.1.

![Diagram of sensitization process](image)

Figure 2.1: The process of sensitization in guinea pigs.

### 2.1.2 Experimental animals

Male Hartley guinea pigs were used for this project. They weighed 300-400 grams during the sensitization period and 400-600 grams at the time of experiments. The animals were housed in an air-conditioned room at 23°C ± 2 and 55 ± 5% humidity with alternating 12 h light/dark cycles and were exposed to food and water ad libitum. The use of the animals for this project was approved by the UBC Animal Care Committee.
2.1.3 Details of methods and models used in this study

2.1.3.1 Sneezing and nose rubbing in conscious guinea pigs in response to allergen challenge

On day 21 post first sensitization, guinea pigs were challenged intranasally either with 2% ovalbumin, 20μl/nostril or 20μl saline/nostril. Drugs were administered before challenge. Sneezing and nose rubbing were observed and counted directly following nasal challenge and for 30 minutes thereafter (Figure 2.2).

2.1.3.2 Measurement of nasal airway pressure and forced inflation pressure in anaesthetized guinea pigs subjected to allergen challenge

Nasal airway pressure and lung inflation pressure were measured in anaesthetized guinea pigs. As previously described (Mizuno et al., 1991) with some modification,
guinea pigs were anaesthetized with pentobarbital (35mg/kg) given intraperitoneally. Subsequently, both carotid artery and jugular vein were isolated and cannulated with polyethylene tubing (PE50) for blood pressure monitoring, and drug delivery, respectively. The trachea was isolated and cannulated with catheters (14G) in both directions, caudally for passive ventilation and lung inflation pressure measurement, and rostrally for nasal airway pressure. For nasal airway pressure measurements, the catheter was passed from the trachea, through the laryngopharynx and the oropharynx, toward the nasopharynx just close to the posterior nares. A ventilation pump was used to deliver a pulsatile air at a rate of 8ml/stroke, 72times/min toward the nasal cavity, and 10ml/kg, 72beats/min toward the lungs. To prevent air leakage during nasal resistance recording, the buccal (oral) cavity was filled with epoxy-soaked cotton and the oesophagus was ligated with surgical thread. Intranasal pressure and lower tracheal pressure were used as indexes of nasal airway pressure and lung inflation pressure respectively, and were recorded via a port off the cannula using a pressure transducer and a Grass-polygraph (Figure 2.3).

Following surgery, guinea pigs were kept for 15 minutes for obtaining baseline blood pressure, nasal airway pressure and lung inflation pressure measures. Then, nasal ventilation pump was disconnected and 0.5ml of 2% ovalbumin challenge infused from the tracheal side toward the nasal cavity for 5 minutes. Thereafter, the nasal ventilation pump was reconnected and the animal was observed for 1 hour (Figure 2.4). This method was previously described by Sakairi 2005 with some modification.
Figure 2.3: Schematic representation of surgical and technical connections for nasal airway pressure, lung inflation pressure and blood pressure measurement.

Figure 2.4: General protocol for nasal airway pressure, lung inflation pressure and cellular infiltration experiments.
2.1.3.3 Attempts to measure nasal secretions and exudation in conscious and anaesthetized guinea pigs subjected to allergen challenge.

Nasal watery secretions can be produced in sensitized guinea pigs after allergen provocation although in most cases its production is not enough for easy evaluation. In an attempt to measure nasal secretions, a pre-weighed cotton swab was rubbed around the anterior naris of sensitized guinea pigs 30-60 minutes after allergen challenge in both conscious and anaesthetized guinea pigs. However the amount collected was not enough to be quantified and evaluated.

We also attempt to quantify exudation in sensitized guinea pigs. We used Evan’s blue and pontamine sky blue dyes. The dyes were administered intravenously in anaesthetized guinea pigs. Thereafter, the guinea pigs were challenged with ovalbumin. After allergen provocation, nasal cavities were perfused with saline at a rate of 0.2-0.25ml/min for 10 minutes. Dye concentration in the collected perfusate was quantified by spectrophotometer using absorbance at 620nm. Dye concentration is proportional to vascular permeability (exudation). However the amount of dye in the perfusate was not enough to be quantified and evaluated.

Therefore because of these reasons nasal secretions and exudation were not evaluated in this study.

2.1.3.4 Measurement of leukocyte infiltration into nasal lavage fluid in anaesthetized guinea pigs subjected to allergen challenge

Nasal cellular infiltration (extravasation) is a characteristic hallmark of allergic rhinitis. Total and differential cell counts in nasal washings were used as indices of
cellular infiltration. As described before, guinea pigs were anaesthetized with pentobarbital and subsequently challenged with ovalbumin. Nasal lavage was collected from guinea pigs one hour post challenge as follows: nasal cavities were washed with 2ml of pre-warmed saline infused from the tracheal side. The recovered saline was collected from the anterior nares. Total and differential cell counts were assessed immediately after sample collection.

Total cell count was assessed using a standard hemocytometer. The ruled area of hemocytometer consists of one large square known as a type A square. The volume of type A square is 0.1 mm$^3$ (Figure 2.5). This area was used to determine the number of cells per milliliter of nasal lavage.

![Schematic representation of a hemocytometer.](image)

Figure 2.5: Schematic representation of a hemocytometer.

After sampling, 50μl of nasal lavage was mixed with 50μl of methylene blue (for cell staining and clarity of counting). The two hemocytometer chambers were filled with
12μl of the mixture per chamber. Leukocytes (white blood cells) were counted under light microscope at power 40X. The mean of the two chambers cells was consider as total cell count. To determine the number of cells per milliliter, the following formula was used:

\[
\text{Number of cells/ml} = \frac{\text{total number of cell counted} \times \text{dilution factor} \times 1000 \ (\text{there are} \ 1000 \ mm^3/ml)}{\text{total volume counted} \ (0.1 \ mm^3)}
\]

To perform a differential cell count, 100μl of nasal lavage was centrifuged using a cytospin (Shandon Cytospin 1) at a speed of 1500 revolutions per minutes for 5 minutes. The sample was then stained with Wright stain solution for 5 minutes. White blood cell type (eosinophil, neutrophil, basophil, monocyte and lymphocyte) was determined based on morphological characteristics. The number of each type of cell was counted under 40X power. Total number of cells per three consistent fields observed was used to reflect the total number of cells.

2.1.3.5 Experimental design and drugs used in the study

The experiments were performed in a randomized double blind fashion to minimize personal bias in data analysis. Guinea pigs were divided into sensitized ovalbumin challenged groups (control), non-sensitized ovalbumin/saline challenged groups (control) and sensitized ovalbumin challenged pre-treated with drug groups.

The actions of drugs currently used clinically or experimentally in the treatment of allergic rhinitis were assessed in terms of their effectiveness in ameliorating sneezing,
nose rubbing, nasal congestion as well as inhibiting cellular infiltration in sensitized guinea pigs. The drugs used in this study were selected based on the previous findings which show their effectiveness in allergic rhinitis in human and animal models. Different drug classes were used including first and second generation antihistamines, leukotriene D4 inhibitor, nitric oxide synthases inhibitor, anti-inflammatory drugs like heparin and corticosteroids. Table 2.1 shows the drugs, their doses and the route of administration used. Drugs were administered intraperitoneally, a 15 minute-period waiting prior to ovalbumin challenge. When they were administered intravenously, a 5 minute-period was used. The doses of the antagonists used in this study were chosen based on literature findings that such doses were effective in blocking their corresponding receptors.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mepyramine</td>
<td>3</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>3</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>Montelukast</td>
<td>10</td>
<td>Intravenous</td>
</tr>
<tr>
<td>L-NAME</td>
<td>10</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Heparin</td>
<td>20</td>
<td>Intravenous</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>20</td>
<td>Intraperitoneal (for sneezing), Intravenously (for nasal airway pressure)</td>
</tr>
</tbody>
</table>

Table 2.1: List of drugs used in the experiments.

The doses of the antagonists for blocking the effects of histamine and acetylcholine on nasal airway pressure and lung inflation pressure study were chosen
based on literature findings that these doses were effective in blocking their corresponding receptors. All drugs were administered intravenously. See Table 2.2.

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Antagonists</th>
<th>10X dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine (2ug-1mg/kg)</td>
<td>M1</td>
<td>Pirenzepine</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>Methoctramine</td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>4-DAMP</td>
</tr>
<tr>
<td>Acetylcholine (2ug-1mg/kg)</td>
<td>M1-M5</td>
<td>Atropine</td>
</tr>
</tbody>
</table>

Table 2.2: List of drugs and doses used in autacoids and neurotransmitters effect on nasal airway pressure and lung inflation pressure study. “10X” is a ten times dose since in this study three doses were used, X, 3X and 10X.

In the histamine and acetylcholine experiments, guinea pigs were divided into 7 groups (n=5 each) (Figure 2.6). All animals received acetylcholine and histamine (2µg-1mg/kg) intravenously as agonists. Three groups, each one of them received three doses (X, 3X, 10X) of one of the muscarinic antagonists [M1 (pirenzepine) (0.09, 0.27 and 0.9mg/kg), M2 (methoctramine) (0.03, 0.09 and 0.3mg/kg) and M3 (4-DAMP) (0.03, 0.09 and 0.3mg/kg)]. One group received atropine at three different doses (0.09, 0.27 and 0.9mg/kg). Three other groups, each one of them received three doses (X, 3X, 10X) of one of the histamine receptors antagonists [(H1 (mepyramine) (0.1, 0.3 and 1mg/kg), H2 (ranitidine) (0.06, 0.18 and 0.6mg/kg) and H3 (thioperamide) (0.05, 0.15 and 0.5mg/kg)]. Figure 2.7 shows the experimental design.
Muscarinic antagonists

- Atropine (5)
- Pirenzepine (5)
- Methoctramine (5)
- 4-DAMP (5)

Histamine receptors antagonist

- Mepyramine (5)
- Ranitide (5)
- Thioperamide (5)

Figure 2.6: Protocol for histamine and acetylcholine experiment. Guinea pigs were divided into seven groups (n=5 per group) based on the antagonist they received.

His: histamine
ACh: acetylcholine
L&H: lower (25% of maximum response and higher doses (75% of maximum response) of His and ACh
Antg: antagonist
X: dose

Figure 2.7: Schematic representation of experimental protocol for histamine and acetylcholine experiment.
2.1.3.6 Data analyses

The data from all the studies were analyzed using parametric statistical tests to determine the probability of rejecting the null hypothesis. In all the studies, the null hypothesis stated that there was no difference between control and treated groups (i.e. all the means are equal). In studies where more than two groups were involved (e.g. non-sensitized control groups, sensitized control group and sensitized pretreated groups), one way analysis of variance (ANOVA) was used to determine whether the variances were different or not, and if they were different (p<0.05), a post-hoc test was used to compare between groups. The post hoc-test used was Bonferroni multiple comparison test and if p>0.05, a less powerful test such as the unpaired student t test was used to compare the means between two selected groups. In studies where two groups were involved and studied over a period of time, a repeated measurement two way ANOVA was used. A probability, p, of less than 0.05 was taken as significant. In each bar in the graphs, the error bar was a standard error of mean.

In experiments where we evaluated the model in terms of allergen responses we tried to use different forms of transformation (e.g. log transformation) to analyze our data, however the outcome did not improve the analysis in terms of the homogenecity of variance, and although the statistical power after transformation was the same as before transformation, we decided to use untransformed raw data for statistical analysis.

Dose ratio method was used to analyze the effectiveness of the antagonists in blocking the actions of acetylcholine or histamine. For nasal airway pressure, $ED_{1.5} - \Delta \text{ANAP (mmHg)}$ was used to measure the potency for acetylcholine, and $ED_{2} - \Delta \text{ANAP (mmHg)}$ for histamine. $[ED_{x} - \Delta \text{ANAP (mmHg)}]$ was the effective dose producing 1.5 or 2
mmHg decrease in nasal airway pressure, from pre-drug value. For lung inflation pressure (LIP), ED\textsubscript{20} + \Delta \text{LIP} (mmHg) was used to measure antagonist shifts for acetylcholine and histamine. \([\text{ED}\textsubscript{20} + \Delta \text{IPP} (\text{mmHg})]\) is the dose producing a 20 mmHg increase in lung inflation pressure from pre-drug value. All the data in the graphs represent mean ± SEM. The relative affinities of the antagonists for muscarinic (M1-5) and histamine (H1-3) receptors were tabulated (Table 2.3) using \(K_B\) values from Leurs et al., 1995 and Bockman et al., 2001. These affinities were used to reflect the possible receptors involved in histamine and acetylcholine-induced changes in nasal airway pressure and lung inflation pressure.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Receptor affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>M1 \approx M2 \approx M3 \approx M4 \approx M5</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>M1 \gg M4 &gt; M3 &gt; M2 \approx M5</td>
</tr>
<tr>
<td>Methoctramine</td>
<td>M2 &gt; M4 &gt; M1 &gt; M5 \gg M3</td>
</tr>
<tr>
<td>4-DAMP</td>
<td>M3 \approx M5 \approx M1 &gt; M4 \gg M2</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>H1 selective</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>H2 &gt;&gt;&gt; H1</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>H3 &gt; H1 \approx H2</td>
</tr>
</tbody>
</table>

Table 2.3: Relative affinities of the antagonists used for muscarinic and histamine receptors

**2.1.3.7 Experimental overview**

In this study different experiments were conducted in order to evaluate the model and drug modification of responses to allergen challenge. All allergic responses, including sneezing, nose rubbing, nasal blockade, and leukocyte infiltration, were
evaluated during the acute phase of allergic reactions. The following experiments were conducted:

i. Assessment of models

The aim of this experiment was to evaluate the effectiveness of sensitization procedures used and to study the effects of allergen challenge on sneezing, nose rubbing, nasal airway pressure, lung inflation pressure, blood pressure and leukocyte infiltration.

ii. An investigation of the receptors involved in mediating nose and lung responses to intravenous acetylcholine and histamine.

During the process of establishing the model, we explored the effects of the autacoid histamine and the neurotransmitter acetylcholine on nasal airway pressure since they are important in allergic rhinitis. Surprisingly when they were administered intravenously, they tended to decrease nasal airway pressure while increasing lung inflation pressure. It is well known that histamine and acetylcholine cause vasodilatation. Applying this concept to the nasal blood vessels, they should be expected to increase nasal airway pressure (Powell et al., 1979; Feigl 1998). Some studies in human being (Tylor-Clark et al., 2005) and animals, namely, dogs (Ruslan et al., 2003) and guinea pigs (Mizutani et al., 1999) have shown that when histamine is given intranasally, nasal airway pressure increases. Other studies in guinea pigs indicated that histamine and acetylcholine introduced topically into the nasal mucosa may increase or decrease nasal airway pressure depending upon the dose (Lung et al., 1987; Lung et al., 1994). Histamine H1 antagonists have been used widely to treat acute symptoms of allergic
rhinitis (Dykewicz et al., 1998). In guinea pigs oral histamine H1 antagonists inhibit histamine-induced nasal congestion (Shigeru et al., 2003).

Therefore this study was conducted in order to determine which receptors are involved in intravenous histamine and acetylcholine-induced decreases in nasal airway pressure in anaesthetized guinea pigs. Furthermore, the study was also aimed to determine the receptors involved in histamine and acetylcholine-provoked increases in lung inflation pressure.

iii. Drug modification of the response to allergen challenge

The aim of this study was to evaluate the effects of therapeutically important drugs on allergen induced sneezing, nose rubbing, nasal airway pressure, and leukocyte infiltration in sensitized guinea pigs. To achieve this aim the following experiments were conducted:

- Effect of antihistamines mepyramine and cetirizine on sneezing, nose rubbing and nasal airway pressure.
- Effect of montelukast on sneezing, nose rubbing, nasal airway pressure and cellular infiltration.
- Effect of L-NAME on nasal airway pressure and cellular infiltration.
- Effect of heparin on sneezing, nose rubbing, nasal airway pressure and cellular infiltration.
- Effect of dexamethasone on sneezing, nose rubbing, nasal airway pressure and cellular infiltration.
3. Results

3.0 Assessment of models

3.0.1 Effectiveness of the sensitization procedures used

During the process of sensitization, the time effect of ovalbumin administration on the frequency of sneezing and nose rubbing in guinea pigs was evaluated. There was a frequency-dependent effect of ovalbumin administration on number of sneezes and nose rubbings (p<0.0001 two way ANOVA). The number of sneezes increased from day 1 to day 16 in the ovalbumin group. No significant effect of saline aerosol/instillation on sneezing frequency was observed (Figure 3.1). The effect of ovalbumin on sneezing frequency achieved significant levels (p<0.01) compared to saline group on days 15 and 16.

![Figure 3.1: Time dependent effect of ovalbumin administration on sneezing frequency in guinea pigs measured during the sensitization period. Days 1 and 7 guinea pigs received 2% aerosolized ovalbumin or saline for 10 minutes in both nostrils. Days 14-16 guinea pigs were boosted with intranasal instillation of 1% ovalbumin or saline 20µl/nostril in both nostrils/day. Sneezes were counted for 30 minutes immediately after aerosol/liquid exposure. * Significant increase in number of sneezes as compared to saline group (p<0.001, Bonferroni multiple comparison test). The data are the means of 10 guinea pigs. Vertical bars represent standard error of mean.](image-url)
In comparison to saline, ovalbumin sensitization increased nose rubbing (p=0.002 two way ANOVA). The significant levels were observed on days 14 (p<0.05), 15 (p<0.01) and 16 (p<0.001) (Figure 3.2).

![Figure 3.2](image_url)

**Figure 3.2:** Time dependent effect of ovalbumin administration on nose rubbing frequency in guinea pigs measured during the sensitization period. Days 1 and 7 guinea pigs received 2% aerosolized ovalbumin or saline for 10 minutes in both nostrils. Days 14-16 guinea pigs received booster doses as intranasal instillation of 1% ovalbumin or saline 20μl/nostril in both nostrils/day. Nose rubs were counted for 30 minutes immediately after aerosol/liquid exposure. * Significant increase in number of nose rubbing as compared to saline group (* p<0.05, ** p<0.01, *** p<0.001, Bonferroni multiple comparison test). The data are the means of 10 guinea pigs. Vertical bars represent standard error of mean.

### 3.0.2 Sneezing and nose rubbing responses to allergen challenge

Ovalbumin challenge in sensitized group significantly increased sneezing frequency (p<0.01) and number of nasal rubs (p<0.001) as compared to non-sensitized groups (Figures 3.3 and 3.4).
Figure 3.3: Effect of ovalbumin challenge on sneezing frequency in sensitized and non-sensitized guinea pigs evaluated day 21 post first sensitization. Guinea pigs received a challenge dose of 2% ovalbumin or saline 20μl/nostril in both nostrils and sneezes were counted for 30 minutes after challenge. * Significant increase in sneeze frequency as compared to non-sensitized ovalbumin challenged guinea pigs (* p<0.01, Bonferroni multiple comparison test). The data are the means of 5 guinea pigs. Vertical bars represent standard error of mean.

Figure 3.4: Effect of ovalbumin challenge on nose rubbing frequency in sensitized and non-sensitized guinea pigs evaluated day 21 post first sensitization. Guinea pigs received a challenge dose of 2% ovalbumin or saline 20μl/nostril to both nostrils and nose rubs were counted for 30 minutes after challenge. * Significant increase in nose rubbing frequency as compared to non-sensitized ovalbumin challenged guinea pigs (* p<0.001, Bonferroni multiple comparison test). The data are the means of 5 guinea pigs. Vertical bars represent standard error of mean.
3.0.3 Changes in nasal airway pressure and lung inflation pressure as well as blood pressure in response to allergen challenge

Ovalbumin challenge in sensitized group significantly increased nasal airway pressure (p<0.05) as compared to non-sensitized groups. No significant change in nasal airway pressure was observed in ovalbumin/saline non-sensitized groups (Figure 3.5).

![Figure 3.5: Effect of ovalbumin challenge on nasal airway pressure in anaesthetized (by pentobarbital) (sensitized and non-sensitized) guinea pigs 23-28 days following initiation of sensitization. Guinea pigs were challenged with 2% ovalbumin or saline at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Nasal airway pressure was measured 30-35 minutes after challenge. The difference between pre-challenge and 30-35 minutes post-challenge values was considered as a change in nasal airway pressure. * Significant increase in nasal airway pressure as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are the means of 5 guinea pigs. Vertical bars represent standard error of mean.]

Simultaneous recording of lung inflation pressure and blood pressure besides nasal airway pressure recording showed no significant changes in these variables when compared between sensitized and non-sensitized groups. Intranasal ovalbumin did not cause significant changes in lung inflation pressure (Figure 3.6).
3.0.4 Leukocyte infiltration responses to allergen challenge

Ovalbumin challenge in sensitized group significantly increased total cell count (p<0.01) as compared to non-sensitized groups. No significant effect of ovalbumin/saline challenge in non-sensitized groups was observed (Figure 3.7). Furthermore, ovalbumin significantly induced both eosinophil (p<0.01) and neutrophil (p<0.05) infiltration in sensitized guinea pigs compared with non-sensitized guinea pigs. No significant effect of ovalbumin was observed on other cell types (Figure 3.8).
Figure 3.7: Effect of ovalbumin challenge on cellular infiltration (total cell count) in anaesthetized (sensitized and non-sensitized) guinea pigs evaluated from nasal lavage collected 60 minutes following 2% ovalbumin or saline challenge at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Cells were counted using hemocytometer under light microscope and the value were expressed as number of cells per milliliter of nasal lavage. * Significant increase in total cell count as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.01, Bonferroni multiple comparison test). n = 5 per group. Vertical bars represent standard error of mean.

3.0.5 Sensitization test

To test whether the guinea pigs were sensitized overall or not, at the end of the experiment, 1mg/kg of ovalbumin was administered intravenously. In sensitized guinea pigs anaphylactic shock occurred. It was characterized by an increase in blood pressure, heart rate and lung inflation pressure immediately (within one minute) after ovalbumin administration. In non-sensitized guinea pigs, no effect of 1mg/kg ovalbumin intravenously administered was observed as exemplified in Figure 3.9.
3.1 An investigation of the receptors involved in mediating nose and lung responses to intravenous acetylcholine and histamine in vivo

3.1.1 Effect of histamine and acetylcholine on nasal airway pressure

Both histamine and acetylcholine produced dose dependent decreases in nasal airway pressure with histamine being more potent ($ED_{0.6-\Delta mm Hg} = 1.8\mu g/kg$ for
histamine and 6.3µg/kg for acetylcholine) and probably with greater efficacy (Emax = 2.4±0.2mmHg for histamine and less than 1.5mmHg for acetylcholine) (Figure 3.10).

### 3.1.2 Effect of histamine and acetylcholine on lung inflation pressure

Acetylcholine and histamine produced dose dependent increases in lung inflation pressure, with histamine being more potent (ED50 = 5±1µg/kg for histamine and 36.3±1µg/kg for acetylcholine) and probably with the same efficacy (Emax = 25.4±0.8mmHg for histamine and 24.9±0.7mmHg for acetylcholine) (Figure 3.11).
Figure 3.10: Dose-response curves for the decrease in nasal airway pressure following intravenous administration of histamine and acetylcholine at doses of μg/kg in non-sensitized guinea pigs. The changes in nasal airway pressure are the difference between pre drug and after drug administration. The data are means of 35 guinea pigs. Vertical bars represent standard error of mean.

Figure 3.11: Dose-response curves for the increase in lung inflation pressure following intravenous administration of histamine and acetylcholine in non-sensitized guinea pigs. The changes in lung inflation pressure are the difference between pre drug and after drug administration. The locators (ED$_{50}$), maximum effects (E$_{max}$) and hill slopes are shown on the graph. The data are mean responses from 35 guinea pigs. Vertical bars represent standard error of mean.
3.1.3 An investigation of receptors involved in histamine and acetylcholine induced changes in nasal airway pressure (NAP) and lung inflation pressure (LIP)

In order to assess the effects of supposedly selective antagonist on the responses to acetylcholine and histamine, the following procedures were used: The initial sensitivity of anaesthetized animals to histamine and acetylcholine were assessed in terms of effects on NAP and LIP. Two suitable test doses of either agonist were chosen from the data used to produce Figures 3.10 and 3.11. After obtaining control responses the first dose of antagonist (x) was administered and the test doses were repeated. This was repeated after 3x and 10x the original dose of antagonist. After the final dose of antagonist an attempt was made to construct a full dose response curve to the particular agonist used. It was presumed that the effector concentration of antagonist in the animal did not change significantly during the test procedure. This procedure resulted in seven groups, each of 5 animals. The data in Figures 3.10 and 3.11 are the accumulated data from the control (no antagonist) data. Each of the 7 groups received one of the seven antagonists (four antimuscarinic and three antihistaminic). The effects of the antagonists were assessed in terms of their ability to shift the responses to the two test doses of each of the two agonists to the left (for NAP, Figure 3.12 for acetylcholine and 3.13 for histamine) and to the right (for LIP, Figure 3.14 for acetylcholine and 3.15 for histamine) and to express a shift as a dose ratio.

In terms of the effects of the various antagonists on acetylcholine and histamine responses Figs 3.12 and 3.13 showed that most antagonists failed to change responses to test doses of agonists. For acetylcholine there was some degree of shift for atropine, pirenzepine and DAMP but none for methoctramine and histamine antagonists (Figure 3.12). On the other hand (Figure 3.13), for histamine the only antagonist that produced
any notable shift was mepyramine. In those cases where any shift occurred it was less than that expected for competitive antagonism (Tables 3.1 and 3.2) on the basis that increasing the antagonist dose from the initial X to 3X and then to 10X would increase the dose ratio (i.e. shift the curves) by a factor of 10 and 30 respectively for competitive antagonism.

In the case of the response of the LIP to acetylcholine and histamine, a better degree of antagonism was seen in some cases. Thus, for acetylcholine, marked shifts were seen with atropine, pirenzepine and DAMP but were non competitive for methoctramine and absent with the histamine antagonists (Figure 3.14). For histamine, marked shift was seen with mepyramine but absent with ranitidine, thioperamide and muscarinic antagonists (Figure 3.15).

Tables 3.1-3.4 summaries results obtained from figures 3.12-3.15, respectively.
Figure 3.12: Dose-response curves for the decrease in nasal airway pressure following intravenous administration of acetylcholine before and after intravenous administration of an antagonist in non-sensitized guinea pigs. A, B, C, D, E, F and G show the effect of atropine (nonselective muscarinic antagonist), perizepine (M1 antagonist), methoctramine (M2 antagonist), 4-DAMP (M3 antagonist), mepyramine (H1 antagonist), ranitidine (H2 antagonist) and thioperamide (H3 antagonist) respectively, on acetylcholine induced decreases in nasal airway pressure. The drugs were administered at three successive doses (X, 3X, and 10X). The X axis is log$_2$. NAP is nasal airway pressure. The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
Figure 3.13: Dose-response curves for the decrease in nasal airway pressure following intravenous administration of histamine before and after intravenous administration of an antagonist in non-sensitized guinea pigs. A, B, C, D, E, and F show the effect of mepyramine (H1 antagonist) and ranitidine (H2 antagonist), thioperamide (H3 antagonist), atropine (nonselective muscarinic antagonist), pernzepine (M1 antagonist), methoctramine (M2 antagonist) and 4-DAMP (M3 antagonist), respectively, on histamine induced decreases in nasal airway pressure. The drugs were administered at three successive doses (X, 3X, and 10X). The X axis is log₂. NAP is nasal airway pressure. The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
### ACh-NAP [ED$_{1.5}$–ΔNAP(mmHg)]

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Summary of actions of antagonist in terms of shifts of dose-response curves</th>
<th>Type of blockade</th>
<th>Receptor involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>A degree of antagonist-induced shift (maximum dose ratio 7) but shift not increased with increasing doses of antagonist</td>
<td>Not obviously competitive</td>
<td>M1-5</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>Slight shift but not clearly related to dose of antagonist</td>
<td>Not competitive</td>
<td>M1, 4</td>
</tr>
<tr>
<td>Methoctramine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No M2, M4</td>
</tr>
<tr>
<td>4-DAMP</td>
<td>Similar pattern of responses to that seen with atropine</td>
<td>Not obviously competitive</td>
<td>M3,M5, M1</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H</td>
</tr>
</tbody>
</table>

Table 3.1: A summary of Figure 3.12. Acetylcholine effects on nasal airway pressure in the presence of different doses of antagonists. Possible receptors blocked by the antagonists are shown based on type of blockade and relative affinities of the antagonists (refer to table 2.3).

### His-NAP [ED$_{2}$–ΔNAP(mmHg)]

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Summary of actions of antagonist in terms of shifts of dose-response curves</th>
<th>Type of blockade</th>
<th>Receptor involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pirenzepine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No M1</td>
</tr>
<tr>
<td>Methoctramine</td>
<td>Slight shift</td>
<td>Not competitive</td>
<td>M2</td>
</tr>
<tr>
<td>4-DAMP</td>
<td>Slight shift (maximum dose ratio 2)</td>
<td>Non-competitive block</td>
<td>M3,5,1</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>Very marked shift by the all doses (dose ratio &gt;100) but the effect of larger doses of antagonist could not be seen due to extend to blockade</td>
<td>Possibly competitive</td>
<td>H1</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H2</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H3</td>
</tr>
</tbody>
</table>

Table 3.2: A summary of Figure 3.13. Histamine effects on nasal airway pressure in the presence of different doses of antagonists. Possible receptors blocked by the antagonists assessed based on the type of blockade and relative affinities of the antagonists (refer to table 2.3).
Figure 3.14: Dose-response curves for the increase in lung inflation pressure following intravenous administration of acetylcholine before and after intravenous administration of an antagonist in non-sensitized guinea pigs. A, B, C, D, E, F and G show the effect of atropine (nonselective muscarinic antagonist), perinzepine (M1 antagonist), methoctramine (M2 antagonist), 4-DAMP (M3 antagonist), mepyramine (H1 antagonist), ranitidine (H2 antagonist) and thioperamide (H3 antagonist) respectively, on acetylcholine induced increases in lung inflation pressure. The drugs were administered at three successive doses (X, 3X, and 10X). The X axis is log$_2$ and the values shown in X axis are anti-log$_2$. The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
Figure 3.15: Dose-response curves for the increase in lung inflation pressure following intravenous administration of histamine before and after intravenous administration of an antagonist in non-sensitized guinea pigs. A, B, C, D, E, and F show the effect of mepyramine (H1 antagonist) and ranitidine (H2 antagonist), thioperamide (H3 antagonist), perinzepine (M1 antagonist), methoctramine (M2 antagonist) and 4-DAMP (M3 antagonist), respectively, on histamine induced decreases in nasal airway pressure. The drugs were administered at three successive doses (X, 3X, and 10X). The X axis is log$_2$ and the values shown in X axis are anti-log$_2$. The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
<table>
<thead>
<tr>
<th>Antagonist</th>
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<th>Type of blockade</th>
<th>Receptor involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>Very marked shift by the all doses (dose ratio &gt;100) but the effect of larger doses of antagonist could not be seen due to extend to blockade</td>
<td>Possibly competitive</td>
<td>M1-5</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>Parallel (?) shift at lower doses of antagonist (maximum dose ratio shift 5)</td>
<td>Possibly competitive</td>
<td>M1</td>
</tr>
<tr>
<td>Methoctramine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No M2, 4</td>
</tr>
<tr>
<td>4-DAMP</td>
<td>Large shift (dose ratio &gt;100) but competitive shift difficult to see</td>
<td>Possibly competitive</td>
<td>M3, 5, 1</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H1</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H2</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H3</td>
</tr>
</tbody>
</table>

Table 3.3: A summary of Figure 3.14. Acetylcholine effect on lung inflation pressure in the presence of different doses of antagonists. The assumed receptors involved is based on blocking property and relative affinities of the antagonists (refer to table 2.3).

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Summary of actions of antagonist in terms of shifts of dose-response curves</th>
<th>Type of blockade</th>
<th>Receptor involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pirenzepine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No M</td>
</tr>
<tr>
<td>Methoctramine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No M</td>
</tr>
<tr>
<td>4-DAMP</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No M</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>Very marked shift by the all doses (dose ratio &gt;100) but the effect of larger doses of antagonist could not be seen due to extend to blockade</td>
<td>Possibly competitive</td>
<td>H1</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H2</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>No antagonist-induced shifts seen</td>
<td>No block</td>
<td>No H3</td>
</tr>
</tbody>
</table>

Table 3.4: A summary of Figure 3.15. Histamine effects on lung inflation pressure in the presence of different doses of antagonists. Possible receptors blocked by the antagonists are stated based on blocking properties and relative affinities of the antagonists (refer to table 2.3).
3.2 Drug modification of responses to allergen challenge

3.2.1 Effect of antihistamines (mepyramine and cetirizine) on acute allergic reactions in sensitized ovalbumin challenged guinea pigs

3.2.1.1 Sneezing and nose rubbing

Intranasal instillation of ovalbumin in sensitized guinea pigs significantly induced sneezing and nose rubbing evaluated during the first 30 minutes following intranasal challenge as compared to non-sensitized guinea pigs. Pretreatment with mepyramine and cetirizine intraperitoneally administered at doses 3mg/kg 15 minutes prior to nasal challenge significantly (p<0.01) inhibited ovalbumin-induced sneezing in sensitized guinea pigs as compared to sensitized untreated guinea pigs (Figure 3.16). In addition, cetirizine at the same dose significantly (p<0.05) attenuated nose rubbing frequency. Mepyramine failed to inhibit ovalbumin-induced nose rubbing in sensitized guinea pigs (Figure 3.17).

3.2.1.1 Nasal airway pressure

Ovalbumin challenge in sensitized guinea pigs significantly caused increases in nasal airway pressure within 30 minutes post ovalbumin challenge as compared to non-sensitized guinea pigs. Cetirizine intraperitoneally administered at a dose of 3mg/kg significantly (p<0.05) attenuated ovalbumin-provoked increases in nasal airway pressure, whereas no effect of mepyramine (3mg/kg) pretreatment on nasal airway pressure was observed (Figure 3.18).
Figure 3.16: Effect of antihistamines; mepyramine 3mg/kg and cetirizine 3mg/kg on sneezing frequency induced by 2% ovalbumin challenge in sensitized guinea pigs. Guinea pigs received a challenge dose of 2% ovalbumin or saline 20μl/nostril in both nostrils and sneezes were counted for 30 minutes after challenge. Drugs were administered ip 15 minutes prior to challenge. * Significant inhibition of sneezing frequency as compared to sensitized untreated guinea pigs (p<0.01, Bonferroni multiple comparison test). # Significant increase in sneezing frequency as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.01, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.

Figure 3.17: Effect of antihistamines; mepyramine 3mg/kg and cetirizine 3mg/kg on frequency of nose rubbing induced by 2% ovalbumin challenge in sensitized guinea pigs. Guinea pigs received a challenge dose of 2% ovalbumin or saline 20μl/nostril in both nostrils and nose rubs were counted for 30 minutes after challenge. Drugs were administered intraperitoneally 15 prior to ovalbumin challenge. * Significant inhibition of nose rubbing frequency as compared to sensitized untreated guinea pigs (p<0.05, Bonferroni multiple comparison test). # Significant increase in nose rubbing frequency as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
3.2.2 Effect of leukotriene D4 receptor antagonist, montelukast, on acute allergic reactions in sensitized ovalbumin challenged guinea pigs

3.2.2.1 Sneezing and nose rubbing

Ovalbumin challenge in sensitized guinea pigs induced sneezing and nose rubbing as compared to non-sensitized guinea pigs. Pretreatment with montelukast 10mg/kg administered intravenously 5 minutes prior to ovalbumin challenge in sensitized guinea pigs failed to attenuate both sneezing and nose rubbing (Figures 3.19 and 3.20).
Figure 3.19: Effect of montelukast 10mg/kg on sneezing frequency induced by 2% ovalbumin challenge in sensitized guinea pigs. Guinea pigs received a challenge dose of 2% ovalbumin or saline 20μl/nostril in both nostrils and sneezes were counted for 30 minutes after challenge. Montelukast was administered intravenously 5 minutes prior to ovalbumin challenge. # Significant increase in sneezing frequency as compared to nonsensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.

Figure 3.20: Effect of montelukast 10mg/kg on nose rubbing frequency induced by 2% ovalbumin challenge in sensitized guinea pigs. Guinea pigs received a challenge dose of 2% ovalbumin or saline 20μl/nostril in both nostrils and nose rubs were counted for 30 minutes after challenge. Montelukast was administered intravenously 5 minutes prior to ovalbumin challenge. # Significant increase in nose rubbing frequency as compared to nonsensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
3.2.2.2 Nasal airway pressure

In sensitized guinea pigs, ovalbumin provocation produced significant increases in nasal airway pressure as compared to non-sensitized guinea pigs. Montelukast 10mg/kg administered intravenously 5 minutes prior to ovalbumin challenge significantly (p<0.01) alleviated increases in nasal airway pressure induced by ovalbumin in sensitized guinea pigs (Figure 3.21).

Figure 3.21: Effect of montelukast 10mg/kg on nasal airway pressure induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with 2% ovalbumin or saline at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Nasal airway pressure was measured 30-35 minutes after challenge. The difference between pre-challenge and 30-35 minutes post-challenge was considered as a change in nasal airway pressure. Montelukast was administered intravenously 5 minutes prior to ovalbumin challenge. NAP: nasal airway pressure. * Significant reduction of nasal airway pressure as compared to sensitized untreated guinea pigs (p<0.01, Bonferroni multiple comparison test). # Significant increase in nasal airway pressure as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.01, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
3.2.2.3 Leukocyte infiltration

Ovalbumin challenge in sensitized guinea pigs induced cellular infiltration during first hour following nasal challenge. This was reflected by increases in total cell count of nasal lavage collected one hour post ovalbumin challenge. No significant change was observed in non-sensitized guinea pigs. Pretreatment with montelukast 10mg/kg iv 5 minutes prior to ovalbumin challenge significantly (p<0.01) reduced total cell count as compared to sensitized ovalbumin challenged group (Figure 3.22).

![Graph showing cellular infiltration induced by ovalbumin challenge and effects of montelukast pretreatment.](image)

Figure 3.22: Effect of montelukast 10mg/kg on cellular infiltration (total cell count) induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with 2% ovalbumin or saline at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Cells were counted using a hemocytometer as number of cells per milliliter of nasal lavage collected 60 minutes after challenge. Montelukast was administered intravenously 5 minutes prior to ovalbumin challenge. * Significant decrease in total cell count as compared to sensitized untreated guinea pigs (p<0.01, Bonferroni multiple comparison test). # Significant increase in total cell count as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.01, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
3.2.3 Effect of N (omega)-nitro-L-arginine methyl ester (L-NAME) on acute allergic reactions in sensitized ovalbumin challenged guinea pigs

3.2.3.1 Nasal airway pressure

In sensitized guinea pigs, ovalbumin provocation produced significant increases in nasal airway pressure as compared to non-sensitized guinea pigs. L-NAME 10mg/kg administered intravenously 5 minutes prior to ovalbumin challenge significantly \((p<0.001)\) inhibited the increase in nasal airway pressure induced by ovalbumin in sensitized guinea pigs (Figure 3.23).

![Figure 3.23: Effect of L-NAME 10mg/kg on nasal airway pressure (NAP) induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with 2% ovalbumin or saline at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Nasal airway pressure was measured 30-35 minutes after challenge. The difference between pre-challenge and 30-35 minutes post-challenge was considered as a change in nasal airway pressure. L-NAME was administered intravenously 5 minutes prior to ovalbumin challenge. * Significant reduction of nasal airway pressure as compared to sensitized untreated guinea pigs \((p<0.001\), Bonferroni multiple comparison test). # Significant increase in nasal airway pressure as compared to non-sensitized ovalbumin challenged guinea pigs \((p<0.05\), Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.](image)
3.2.3.2 Leukocyte infiltration

Ovalbumin challenge in sensitized guinea pigs induced cellular infiltration in the first hour following nasal challenge. This was reflected by increases in total cell count of nasal lavage collected one hour post ovalbumin challenge. No significant change was observed in non-sensitized guinea pigs. Pretreatment with L-NAME 10mg/kg intravenously administered 5 minutes prior to ovalbumin challenge failed to reduce total cell count within one hour post ovalbumin challenge in sensitized guinea pigs (Figure 3.24).

Figure 3.24: Effect of L-NAME 10mg/kg on cellular infiltration (total cell count) induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with 2% ovalbumin or saline at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Cells were counted using hemocytometer under light microscope and the value were expressed as number of cells per milliliter of nasal lavage collected 60 minutes after challenge. L-NAME was administered intravenously 5 minutes prior to ovalbumin challenge. # Significant increase in total cell count as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
Ovalbumin challenge in sensitized guinea pigs significantly stimulated eosinophil infiltration within first hour after ovalbumin challenge as compared to non-sensitized guinea pigs. This was reflected by an increase in eosinophils in nasal lavage sample detected by differential cell count. No significant increase in neutrophils, basophils, monocytes and lymphocytes was detected when compared between sensitized and non-sensitized guinea pigs. Pretreatment with L-NAME 10mg/kg intravenously administered 5 minutes prior to ovalbumin challenge significantly (p<0.001) induced increases in neutrophil infiltration in treated group as compared to untreated groups (Figure 3.25).

Figure 3.25: Effect of L-NAME 10mg/kg on cellular infiltration (differential cell count) induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with 2% ovalbumin or saline at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Cells were counted under light microscope at 40X power and total cells counted in three consistent fields were used to express the changes. L-NAME was administered intravenously 5 minutes prior to ovalbumin challenge. * Significant increase in neutrophil number as compared to non-sensitized ovalbumin challenged guinea pigs (* p<0.001, Bonferroni multiple comparison test). # Significant increase in eosinophil number as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.01, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
3.2.4 Effect of heparin on acute allergic reactions in sensitized ovalbumin challenged guinea pigs

3.2.4.1 Sneezing and nose rubbing

Ovalbumin challenge in sensitized guinea pigs induced sneezing and nose rubbing as compared with non-sensitized. Pretreatment with heparin 20mg/kg administered intravenously 5 minutes prior to ovalbumin challenge failed to attenuate both sneezing and nose rubbing (Figures 3.26 and 3.27).

Figure 3.26: Effect of heparin 20mg/kg on sneezing frequency induced by 2% ovalbumin challenge in sensitized guinea pigs. Guinea pigs received ovalbumin, or saline, at 20μl/nostril in both nostrils. Sneezes were counted for 30 minutes after challenge. Heparin iv was administered 5 minutes prior to ovalbumin. # Significant increase in sneezing frequency as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
Figure 3.27: Effect of heparin 20mg/kg iv on nose rubbing induced by 2% ovalbumin challenge in sensitized guinea pigs. Guinea pigs received ovalbumin, or saline, at 20μl/nostril in both nostrils. Nose rubs were counted for 30 minutes after challenge. Heparin was administered iv 5 minutes prior to challenge. # Significant increase in nose rubbing frequency as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.

### 3.2.4.2 Nasal airway pressure

In sensitized guinea pigs, ovalbumin provocation produced significant increases in nasal airway pressure as compared with non-sensitized guinea pigs. Heparin 20mg/kg iv 5 minutes prior to ovalbumin challenge significantly (p<0.01) alleviated the increase in nasal airway pressure induced by ovalbumin challenge in sensitized guinea pigs (Figure 3.28).
### 3.2.4.3 Leukocyte infiltration

Ovalbumin challenge in sensitized guinea pigs induced cellular infiltration during the first hour following nasal challenge. This was reflected by increases in total cell counts in the nasal lavage collected one hour post ovalbumin challenge. No significant change was observed in non-sensitized guinea pigs. Pretreatment with heparin 20mg/kg significantly (p<0.01) reduced total cell count as compared with sensitized ovalbumin challenged group (Figure 3.29)
3.2.5 Effect of dexamethasone on acute allergic reactions in sensitized ovalbumin challenged guinea pigs

3.2.5.1 Sneezing and nose rubbing

Intranasal instillation of ovalbumin in sensitized guinea pigs significantly induced sneezing and nose rubbing in the first 30 minutes following intranasal challenge. Pretreatment with dexamethasone (20mg/kg ip) 15 minutes prior to nasal challenge significantly \( (p<0.001) \) inhibited ovalbumin-induced sneezing in sensitized guinea pigs as
compared with sensitized non treated guinea pigs (Figure 3.30). However, dexamethasone failed to attenuate ovalbumin induced nose rubbing (Figure 3.31).

![Bar graph showing sneezes per 30 minutes](image)

**Figure 3.30:** Effect of dexamethasone 20mg/kg ip on number of sneezes induced by ovalbumin challenge in sensitized guinea pigs. Guinea pigs received ovalbumin, or saline, 20µl/nostril in both nostrils and sneezes were counted for 30 minutes after challenge. Dexamethasone administered 15 minutes prior to ovalbumin challenge. * Significant reduction in number of sneezes as compared to untreated sensitized guinea pigs (p<0.001, Bonferroni multiple comparison test). # Significant increase in sneezing frequency as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.

### 3.2.5.2 Nasal airway pressure

Nasal airway pressure increased following ovalbumin challenge in sensitized guinea pigs. Dexamethasone 20mg/kg administered intravenously 5 minutes prior to ovalbumin challenge failed to prevent ovalbumin induced increases in nasal airway pressure (Figure 3.32).
Figure 3.31: Effect of Dexamethasone 20mg/kg administered intraperitoneally on nose rubbing frequency induced by ovalbumin challenge in sensitized guinea pigs. Guinea pigs received ovalbumin, or saline, 20μl/nostril in both nostrils and nose rubs were counted for 30 minutes after challenge. Dexamethasone was administered 15 minutes prior to ovalbumin challenge. # Significant increase in nose rubbing frequency as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, unpaired student t-test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.

Figure 3.32: Effect of dexamethasone 20mg/kg iv on nasal airway pressure (NAP) induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with ovalbumin, or saline, at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Nasal airway pressure was measured 30-35 minutes after challenge. The difference between pre-challenge and 30-35 minutes post-challenge was considered as a change in nasal airway pressure. Dexamethasone was administered 5 minutes prior to ovalbumin challenge. # Significant increase in nasal airway pressure as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, Bonferroni multiple comparison test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.
3.2.5.3 Leukocyte infiltration

There was an increase in leukocyte infiltration following ovalbumin challenge in sensitized guinea pigs. This was reflected by increases in total cell count of nasal lavage collected one hour post ovalbumin challenge. No significant change was observed in non-sensitized guinea pigs. Pretreatment with dexamethasone (20mg/kg iv) 5 minutes prior to ovalbumin challenge failed to reduce total cell (Figure 3.33).

Figure 3.33: Effect of dexamethasone 20mg/kg administered intravenously on cellular infiltration (total cell count) induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with ovalbumin, or saline, at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Cells were counted using hemocytometer under light microscope and the value were expressed as number of cells per milliliter of nasal lavage collected 60 minutes after challenge. Dexamethasone was administered 5 minutes prior to ovalbumin challenge. # Significant increase in total cell count as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, unpaired student t-test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.

Ovalbumin challenge in sensitized guinea pigs significantly stimulated eosinophil infiltration within first hour after ovalbumin challenge as compared to non-sensitized guinea pigs. This was reflected by an increase in eosinophils in nasal lavage sample.
detected by differential cell count. No significant increase in neutrophils, basophils, monocytes and lymphocytes was detected when compared between sensitized and nonsensitized guinea pigs. Pretreatment with dexamethasone 20mg/kg intravenously administered 5 minutes prior to ovalbumin challenge did not cause significant changes in differential cell count when compared with untreated groups although there is a none statistically significant increase in neutrophils (Figure 3.34).

![Figure 3.34: Effect of dexamethasone 20mg/kg intravenously administered on cellular infiltration (differential cell count) induced by ovalbumin challenge in anaesthetized, sensitized guinea pigs. Guinea pigs were challenged with 2% ovalbumin or saline at a volume of 0.5ml infused to the nasal cavities for 5 minutes. Cells were counted under light microscope at 40X power and total cells counted in three consistent fields were used to express the changes. Dexamethasone was administered intravenously 5 minutes prior to ovalbumin challenge. # Significant increase in eosinophil number as compared to non-sensitized ovalbumin challenged guinea pigs (p<0.05, unpaired student t-test). The data are means of 5 guinea pigs. Vertical bars represent standard error of mean.](image-url)
Tables 3.5 and 3.6 summarise the findings of the above conducted studies.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Allergic responses</th>
<th>Possible target mediator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sneezing</td>
<td>Nose rubbing</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>Inhibited</td>
<td>No effect</td>
</tr>
<tr>
<td>Cetirizine</td>
<td>Inhibited</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Montelukast</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>L-NAME</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>Heparin</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Inhibited</td>
<td>No effect</td>
</tr>
</tbody>
</table>

Table 3.5: A summary of drug modification of the response to allergen challenge experiments.
Table 3.6: A data summary of changes in nasal airway pressure (NAP), lung inflation pressure (LIP), main arterial blood pressure (MAP) and heart rate (HR) in various control and treated guinea pigs measured 30 minutes post challenge. NSC: non-sensitized saline challenged, NOC: non-sensitized ovalbumin challenged, SOC: sensitized ovalbumin challenged. \(^a\) is statistical significant from \(^b\) The values represent mean ± SEM of \(n = 5\).
4 Discussion

4.0 Value of the models in terms of responses to allergen challenge and relevance to clinical rhinitis with special reference to effects of histamine and acetylcholine.

Guinea pigs have been the species of choice for the evaluation of chemical-related respiratory allergy, primarily because it is possible in this species to elicit and measure with relative ease challenged-induced inflammatory reactions that resemble in some ways the acute clinical manifestations of human allergic rhinitis. In this study the procedures used to sensitized guinea pigs were effective. Sensitized guinea pigs produced acute allergic responses after allergen provocation characterized by sneezing, nose rubbing, increase in nasal airway pressure and eosinophil infiltration. In clinical situations, people who suffer from allergic rhinitis suffer from symptoms of sneezing, itching, nasal secretions, nasal congestion in addition to eosinophil infiltration. The currently established model can be used to accurately compare effectiveness of drugs currently used in clinic in addition to further investigation of new drugs. In addition, this model shows no effect of intranasal allergen challenge on lung inflation pressure which may indicate that intranasal allergen challenge specifically triggers nasal inflammatory responses without affecting those of lower respiratory tract. Furthermore, sensitized guinea pigs produced anaphylactic shock immediately after intravenous administration of 1mg/kg ovalbumin. The response was characterized by rapid increase in blood pressure, heart rate and lung inflation pressure. This reflects systemic sensitization following topical nasal sensitization.

Histamine is thought to be a main mediator in the acute inflammatory reaction of allergic rhinitis (Passalacua et al., 2000). Histamine H1 antagonists are commonly used
to treat the acute phase symptoms including sneezing, itchiness and nasal secretions (Dykewicz et al., 1998). However their action in ameliorating nasal congestion is poor (Nabe et al., 2001). Some studies have shown that histamine H1 antagonists reduced nasal congestion (Dykewicz et al., 1998). Other studies on the other hand, concluded there was no effect of H1 antagonists in decreasing nasal airway pressure (congestion) (Nabe et al., 2001) although when histamine was applied topically into the nasal mucosa, it causes increases in nasal resistance (Mizutani et al., 1999; Ruslan et al., 2003; Tylor-Clark et al, 2005). Other studies have shown that a combination of H1 and H3 histamine receptor antagonists may reduce histamine-provoked increases in nasal airway pressure (Tylor-Clark et al., 2005). It also has been shown that, depending on the dose given intranasally, histamine may cause an increase or decrease in the nasal airway pressure (Lung et al., 1987).

There are no published studied on the effect of histamine on nasal airway pressure when it is given intravenously. In our study we have found that when histamine is administered intravenously to guinea pigs, it causes decreases in the nasal airway pressure in a dose-dependent fashion. Mepyramine, possibly competitively, antagonized histamine induced-decreases in nasal airway pressure, whereas no effect of ranitidine and thioperamide were observed. This may suggest the involvement of histamine H1 receptors in this action. Pirenzepine did not prevent histamine-induced decreases in nasal airway pressure. No muscarinic M1 receptors were involved in histamine action. However, methoctramine (to some extent selective on M2 receptors but has very low affinity to M3 receptors) and 4-DAMP (same affinity on M1, 3, and 5 receptors) produced a slight shift in histamine action. This may suggest the involvement of M2 and
perhaps M5 receptors. Taking all this into account, it may lead to the conclusion that histamine-induced decreases in nasal airway pressure is due its action on H1, M2 and perhaps M5 receptors.

The reason why topical application causes increases in nasal resistance, whereas intravenous application causes decreases in the nasal resistance, is not well understood. Results from previous studies have shown that there is a local regulation of norepinephrine transmission in nasal mucosa involving excitatory H1 receptors, inhibitory H2 receptors and inhibitory muscarinic receptors which can be facilitated by sensory stimulation with histamine. Histamine regulation may vary with a balance between the excitatory and inhibitory effects. Histamine in the high concentrations seen in cases of nasal allergy may shift this balance causing increased norepinephrine release. At low concentrations, however, norepinephrine release is inhibited. Norepinephrine causes nasal vasoconstriction that lead to decrease in the nasal airway pressure (Kubo et al., 1989). Study in rabbits showed that H1-receptors mediate negative inotropic effects and vasoconstriction, whereas H2-receptors are responsible for positive inotropic and chronotropic effects and vasodilatation (Sakai 1980).

Although anticholinergic drugs are known to decrease mucous secretion in allergic rhinitis (Lanny et al., 2002), few studies have shown their role in congestion. One study in dogs indicated that acetylcholine given intraarterially may increase or decrease nasal airway pressure, depending on the dose administered, probably via a dose-dependent differential action on different components of the nasal vascular bed (Lung et al., 1994). In our study we found that acetylcholine administered intravenously caused a decrease in the nasal airway pressure in a dose-dependent manner. Methoctramine failed
to prevent acetylcholine-induced decreases in nasal airway pressure which may suggest that no muscarinic M2 and M4 receptors were involved, whereas pirenzepine and 4-DAMP, non-competitively, slightly prevented acetylcholine action. Since 4-DAMP acts equally on M1, M3 and M5 receptors, and pirenzepine acts mainly on M1 receptor, our best guess for the receptor involved in this action would be muscarinic M1 receptors. None of the antihistamines prevented acetylcholine-induced decreases in nasal airway pressure. This may lead to conclusion that acetylcholine action to decrease nasal airway pressure does not involve histamine receptors.

The main focus in our study was nasal airway pressure. However, we also studied the role of muscarinic and histamine receptors on changes in lung inflation pressure. Although we did not study muscarinic 4 and 5 receptors, the results were in general agreement with the literature. Lung inflation pressure increased in a dose-dependent fashion after acetylcholine and histamine were administered. Acetylcholine and histamine bind to receptors in airway smooth muscle cells causing increases in global intracellular calcium release. Calcium-calmodulin complex binds to myosin light chain resulting in phosphorilation of myocin light chain and eventually smooth muscle contraction, and thus bronchoconstriction which leads to increase in lung inflation pressure. Methoctramine did not prevent acetylcholine-induced increases in lung inflation pressure, hence no involvement of M2 and M4 receptors. On the other hand, pirenzepine (has higher affinity to M1 receptors than other muscarinic receptors) and 4-DAMP (shows equal affinity to M1, 3 and 5 receptors) presumably competitively blocked acetylcholine-induced increases in lung inflation pressure. No effects of antihistaminics were observed. Thus, acetylcholine acts mainly on M1 receptors to cause increases in lung inflation pressure.
pressure. Mepyramine, presumably competitively, blocked histamine induced-increases in lung inflation pressure. However, neither the muscarinic antagonist nor ranitidine and thioperamide blocked histamine effect. The most probably receptor involved in the histamine action was H1.

4.1 Possible clinical relevance of model responses to clinical and experimental drugs

4.1.1 Effect of antihistamines on sneezing, nose rubbing and nasal airway pressure in sensitized ovalbumin challenged guinea pigs

Histamine, a natural body constituent, is a low-molecular-weight amine synthesized from L-histidine exclusively by histidine decarboxylase, an enzyme that is expressed in cells throughout the body, including central nervous system neurons, gastric-mucosa parietal cells, mast cells, and basophils (Schayer 1956; Fujikura et al., 2001; Akdis et al., 2003). Histamine plays a pivotal role in allergic inflammation. Nasal challenge with histamine causes sneezing, pain, pruritus, rhinorrhea and nasal blockage (Doyle et al., 1990). In addition, histamine levels in nasal lavages increase following allergen challenge (Naclerio et al., 1985).

Histamine is one of the major chemical mediators triggering the symptoms of rhinitis, including sneezing, nasal itching and rhinorrhea in allergic rhinitis patients, although it is not extensively involved in the increase in nasal airway pressure in allergic rhinitis patients as shown by the limited effect of antihistamines on nasal congestion (Skoner et al., 1994; Numata et al., 1999). Most of the effects of histamine in allergic disease occur through H1 receptors (Schmelz et al., 1997; Schneider et al., 2002; Akdis et
HI-antihistamines act as inverse agonists that combine with and stabilize the inactive form of the H1-receptor, shifting the equilibrium toward the inactive state (Bakker et al., 2001; Leurs et al., 2002). H1-antihistamines (second generation antihistamines being mostly used currently for their minimal sedative effect), either administered orally or applied topically to mucosal surfaces, are the most commonly used first-line medications for both seasonal and perennial allergic rhinitis (Bousquet et al., 2001).

With the regard to their mechanism of action, H1-receptors are coupled to phospholipase C, and their activation leads to formation of inositol-1,4,5-trisphosphate (IP3) and diacylglycerols from phospholipids in the cell membrane; IP3 causes a rapid release of calcium from the endoplasmic reticulum. Diacylglycerol and calcium activate protein kinase C, while calcium activates calcium/calmodulin-dependent protein kinases and phospholipase A2 in the target cell to generate the characteristic response.

Cetirizine and mepyramine are both highly selective for H1 receptors as compared to H2, H3 (Bernheim et al., 1991; Leurs et al., 1995). In addition, cetirizine is highly selective for H1 receptors as compared to serotonergic, adrenergic and muscarinic receptors (Gillard et al., 2003). In this study, mepyramine (first generation H1-antihistamine) and cetirizine (second generation H1-antihistamine) significantly inhibited sneezing frequency during the acute phase of allergic reactions in guinea pigs. In addition, cetirizine, but not mepyramine significantly, inhibited nose rubbing. These results support what has been postulated about the important role of histamine in induction of sneezing during allergic rhinitis and that antihistamines inhibit antigen-induced sneezing (Narita et al., 1997; Fujita et al., 1999; Yamasaki et al., 2001).
(pruritus) is commonly defined in humans as an unpleasant sensation of the superficial layers of the skin (Shelley et al., 1957) provoking the desire to scratch (Ekblom 1995). It is a common clinical condition that can be associated with cutaneous (e.g. atopic eczema, contact dermatitis) or systemic (e.g. chronic renal failure) disease. Itch is difficult to study objectively in man and there are currently few reliable animal models of itch. In addition, the role of histamine in induction of nose rubbing in guinea pig models of allergic rhinitis has not been shown clearly, although some studies reported that antihistamines may inhibit nose rubbing (Zhao et al., 2005). Histamine acts on H1-receptor on nerve endings, resulting in central neuronal reflexes, leading to sneezing and itching. Mepyramine failed to alleviate nasal scratching. It has been postulated that classical H1 receptor antihistamines (e.g. mepyramine) are not effective in alleviating many chronic pruritic conditions (Greaves 1997). Furthermore, it has been shown in a murine model of itching that histamine mediates this symptom through its action on histamine H4 receptors (Bell et al., 2004). Therefore, histamine released after allergen provocation acts also on H4 receptors mediating itching sensation suggesting why mepyramine failed to inhibit these responses. However, cetirizine attenuated nose rubbing, suggesting that this agent has additional pharmacological actions. In fact, cetirizine has a number of antiallergic, anti-inflammatory properties that appear to be independent of its H1-blockade activity (Assanasen et al., 2002), and these may account for its effectiveness in inhibition of nose rubbing.

Our results are consistence to what has been found in studies conducted in allergic rhinitic guinea pigs, in that mepyramine ameliorates sneezing frequency (Mizutani et al., 2003; Nabe et al., 2001; Narita et al., 1993). Additionally, clinical
studies have shown that cetirizine is effective in inhibition of sneezing (Allegra et al., 1993; Baroody et al., 1989).

In our study, cetirizine, but not mepyramine, significantly inhibited ovalbumin-induced increases in nasal airway pressure during the acute phase of allergic rhinitis in guinea pigs. Preclinical studies in animal models of allergic rhinitis have shown that histamine has a role in induction of nasal airway pressure especially during the acute phase of the disease (Mizutani et al., 1999; McLeod et al., 2002; Bockman et al., 2002; Mizutani et al., 2003). In addition, not all antihistamines alleviate the increase in nasal airway pressure induced by antigen in animal models of rhinitis (Nabe et al., 2001; Mizutani et al., 2003; Sakairi et al., 2005). Mepyramine failed to alleviate the increase in nasal airway pressure, mimicking what has been found in previous studies in guinea pig model of allergic rhinitis (Terasawa et al., 1988; Narita et al., 1993; Mizutani et al., 1999; McLeod et al., 2002; Bockman et al., 2002; Mizutani et al., 2003). On the other hand, cetirizine prevented nasal airway pressure increase after allergen provocation. Murata has shown that cetirizine inhibits antigen-induced nasal resistance in guinea pigs (Murata et al., 1997).

The reason behind this contradiction in antihistamine effects on nasal airway pressure is not well understood. It has been postulated that H1-antihistamines have multiple effects on the allergic inflammatory response. It is equally clear that these antiallergic effects are not uniformly shared among all drugs of this class. Furthermore, data from in vitro, in vivo, and ex vivo studies suggest that second-generation antihistamines (e.g. cetirizine) have a number of antiallergic, anti-inflammatory properties that appear to be independent of their H1-blockade activity (Assanasen et al., 2003).
2002). Moreover, a number of antihistamines have been shown to relieve nasal congestion, an effect possibly related to the combination of the direct and potent antihistaminic effects and their well-established anti-inflammatory properties. This is especially true for one of the best-investigated and widely used second-generation antihistamines, cetirizine, which is a 50/50 racemate mixture of levocetirizine and dextrocetirizine. The predecessor of levocetirizine has been shown to exhibit a number of actions that determine its anti-inflammatory properties, including inhibition of eosinophil accumulation, induced by pollen or platelet-activating factor (PAF) (Fadel et al., 1990; Walsh 2000), inhibition of eosinophil migration to, and infiltration, at the sites of allergic challenge (Fadel et al., 1990; Charlesworth et al., 1989), reduction of ICAM-1 (intercellular adhesion molecule 1) expression (Fasce et al., 1996), and inhibition of monocytes and T lymphocytes (Jinquan et al., 1995). The potent inhibitory effect of levocetirizine on the H1 receptor, histamine-induced inflammation, its ability to control vascular dilation and plasma exudation, as well as direct evidence from nasal provocation tests, confirm the potential of this novel antihistamine to control the early-phase reactions in the pathogenesis of nasal congestion. Thus, it could be these additional anti-inflammatory properties of cetirizine behind its additional role in nasal airway pressure alleviation, the property which is not observed in mepyramine.

4.1.2 Effect of leukotriene D4 receptor antagonist, montelukast on sneezing, nose rubbing, nasal airway pressure and leukocyte infiltration in sensitized ovalbumin challenged guinea pigs

The cysteinyl leukotrienes (cysLTs) produce their biological actions by binding and activating specific receptors located on the cell membranes of target cells. Two
subtypes of cysLT receptor have been pharmacologically characterized cysLT₁ and cysLT₂ (Coleman et al., 1995; Nicosia et al., 1999). Montelukast has twice potency than LTD₄ to cysLT₁ receptor. Montelukast inhibits the binding of LTD₄ to this receptor (Aharony 1998). In our study, montelukast significantly inhibited ovalbumin-induced increases in nasal airway pressure in guinea pigs during the acute phase of allergic rhinitis. Furthermore, montelukast also significantly inhibited cellular infiltration (correlated with reduction in total cell count) during the acute phase of antigen-induced rhinitis. However the drug failed to alleviate sneezing and nose rubbing after allergen challenge. This is the first study to demonstrate the effect of montelukast on such allergic symptoms using a guinea pig model of antigen-induced rhinitis. In terms of nasal airway pressure, our results are consistent with previous studies in guinea pig model of allergic rhinitis. It has been demonstrated (using different cysLT₁ receptor antagonists) that cysLTs (especially LTD₄) are significantly involved in the development of nasal congestion (Shizawa et al., 1997; Kaise et al., 1998; Fujita et al., 1999; Mizutani et al., 2001; Yamasaki et al., 2001; Mizutani et al., 2003).

Cysteinyl LTs such as leukotriene C₄, D₄ and E₄ (LTC₄, LTD₄ and LTE₄) are released from various inflammatory cells including mast cells and eosinophils. These mediators are able to increase nasal blood flow (Bisgaard et al., 1986). A recent human study has shown that nasal provocation by LTD₄ can induce nasal obstruction, as indicated by a prolonged increase of nasal airway pressure (Okuda et al., 1988). Furthermore, it has been demonstrated that nasal congestion in the early-phase is accompanied by a significant increase of cysteinyl LTs in nasal lavage fluid from patients with allergic rhinitis (Naclerio et al., 1991b). In addition, LTD₄ has been demonstrated to
dilate nasal blood vessels, which can be related to hyperproduction of nitric oxide through cysteiny1 LT1-receptor activation. These findings suggest the hypothesis that cysteiny1 LTs play an important role in allergic rhinitis, especially in nasal obstruction due to edema of the nasal mucosa membrane (Mizutani et al., 2001).

Besides alleviating nasal congestion, montelukast reduces eosinophil infiltration (reduction of the total cell count after allergen provocation) in the nasal mucosa as associated with the early phase (eosinophils are involved in the acute phase of allergic reactions in a guinea pig model of rhinitis (Imai et al., 2000)) of allergic rhinitis. This result is consistent with previous findings that LTD4 causes significant eosinophil infiltration in nasal mucosa which persists for up to 24 h after the topical challenge in guinea pigs (Fujita et al., 1997). Moreover, a recent report that cysteiny1 LTs induces eosinophil infiltration in lower airway in human (Laitinen et al., 1993) and guinea pigs (Underwood et al., 1996) also supports the suggestion that cysteiny1 LTs contribute to eosinophil infiltration. However, in spite of its potent chemotactic activity (as low as \(10^{-10}\) M) in human eosinophils (Spada et al., 1994), LTD4 up to 10 \(\mu\)M had no apparent chemotactic activity in guinea pig eosinophils in vitro (Fujita et al., 1999). Therefore, its action is not attributable to direct chemotactic activity in guinea pig eosinophils. It is speculated that cells other than eosinophils produce eosinophil chemoattractant(s) in response to LTD4. Thromboxane A2 may be one of the candidate mediators, since it can be released from tissues by LTD4 stimulation (Cheng et al., 1990) and its involvement has been suggested in nasal eosinophil migration in guinea pigs (Narita et al., 1996).

In contrast to the prevention of nasal airway pressure increases, sneezing and nose rubbing in the early phase were not reduced by montelukast. In recent clinical studies,
montelukast prevents symptoms scores in patients with perennial allergic rhinitis (Patel et al., 2005) and seasonal allergic rhinitis (Chervinsky et al., 2004). However, our result is consistent with the findings that LTD4 does not induce sneezing in human (Okuda et al., 1988) and guinea pigs (Fujita et al., 1997b). Thus, it appears that cysteiny1 LTs play a minor direct role in sneezing and nose rubbing. Nevertheless, the possibility that cysteiny1 LTs participate indirectly in sneezing and nasal itchings via an increase in hypersensitivity to specific and/or non-specific stimulus may not be totally excluded. Recently, it has been suggested that LTD4 enhances the responsiveness of capsaicin-sensitive afferent fibers in the guinea pig airway (Undem, 1993).

The results of the present study support the involvement of cysteiny1 LTs in allergic rhinitis, especially in oedema of nasal membrane mucosa causing nasal obstruction. Cysteiny1 LT receptor antagonists, such as montelukast, thus have therapeutic potential in the treatment of allergic rhinitis.

4.1.3 Effect of L-NAME on nasal airway pressure and leukocyte infiltration in sensitized ovalbumin challenged guinea pigs

Nitric oxide, a free radical gas, is an endogenous cell-signaling molecule implicated in a wide range of physiological and pathophysiological events in numerous cell types and processes, including the cardiovascular, immune and nervous systems. Endogenous nitric oxide is produced from the amino acid L-arginine by a family of enzymes called nitric oxide synthases. There are three isoforms of nitric oxide synthases (NOS); constitutive NO synthase which has two isoforms, neural NOS (NOS-1) and endothelial NOS (NOS-3), which have been reported to be expressed in nerve cells and
endothelial cells in arterioles, sinusoid vessels and capillary bed, respectively. Another isoform is inducible NOS (NOS-2), the expression of which in epithelial cells, submucosal glands and inflammatory cells in the nasal mucosa of allergic rhinitis patients is more marked than that of subjects without nasal allergy (Hanazawa et al., 1993; Kawamoto et al., 1998).

Nitric oxide (NO) is a powerful vasodilator that modulates systemic vascular tone (Rees et al., 1989). In addition, NO can cause tissue injury by contributing to the generation of highly reactive oxygen radicals (Beckman et al., 1991). One study showed that a large amount of NO originating from the paranasal sinuses was continuously produced in the nasal cavities of healthy subjects (Lundberg et al., 1995). Furthermore, it has been reported that the NO concentration in exhaled air was elevated in patients with allergic rhinitis compared to that in normal subjects (Arnal et al. 1997; Kharitonov et al. 1997).

NO is known to cause marked vasodilatation by producing an increase in intracellular cyclic GMP level, and to control systemic vascular tone (Imai et al., 2001). There is evidence that nitric oxide is involved in pathogenesis of allergic rhinitis. High levels of nitric oxide have been detected in patients with allergic rhinitis (Arnal et al. 1997; Kharitonov et al. 1997). Furthermore, nitric oxide causes vasodilatation and glandular secretion (Baraniuk 1997). The contribution of NO to the antigen-induced increase in specific airway resistance was evaluated using L-NAME, a non-specific nitric oxide synthase inhibitor. The nasal airway pressure elevations at the early phase induced by antigen challenge was suppressed by L-NAME, given iv 30 minutes before the specific airway resistance measurement, and the suppression was greater than 95%. Our
results support previous finding that NO is involved in nasal airway pressure increase after allergen challenge in guinea pig models of allergic rhinitis (Imai et al., 2001; Mizutani et al., 2001; Bockman et al., 2002; Zhao et al., 2005). Furthermore, L-NAME failed to attenuate eosinophil infiltration after allergen challenge, suggesting that this drug alleviates nasal congestion by a mechanism that might not involve eosinophil inhibition.

The inhibitory action of L-NAME on the early nasal airway pressure increases is assumed to be mainly due to the direct suppression of nasal vasodilatation. Kageyama et al., 1997 reported that NO induces microvascular leakage as a result of indirect vasodilatation with direct endothelial contraction at the site of leakage. Thus, it could be that L-NAME inhibits the specific airway resistance increase by attenuating NO-induced plasma extravasation, in addition to suppressing vasodilatation.

The expression of which of NOS isoforms during allergic rhinitis is not very clear. One study has reported that endothelial NOS is expressed in sensitized guinea pig mucosal tissue (Zhao et al., 2005). In other study, it has been shown that constitutive NOS (both neuronal and endothelial NOS), but not inducible NOS, is involved in nitric oxide production after allergen provocation in sensitized guinea pigs (Imai et al., 2001).

In the current study, L-NAME caused neutrophil infiltration. There is a growing body of evidence that NO from both endogenous and exogenous sources limits leukocyte recruitment into normal and inflamed vessels. Inhibition of endogenous NO with L-NAME promotes leukocyte adhesion in various vascular beds and species (Kubes et al., 1991; Kubes et al., 1993; Ma et al., 1993). Thus, endogenous NO is an important homeostatic regulator of leukocyte adhesion in postcapillary venules. Exposure of
venular endothelium and neutrophils for 60 minutes to NO inhibitors (e.g. L-NAME) \textit{in vitro} does not induce neutrophil–endothelial cell interactions (Niu et al., 1994). This observation raises the possibility that some cell types were missing in the sample \textit{in vitro} system. Mast cells are closely apposed to the vasculature and upon activation induce neutrophil-endothelium interactions (Gaboury et al., 1995). Inhibition of NO synthesis causes mast cell degranulation \textit{in vivo}, and this event could conceivably induce leukocyte recruitment. Indeed, L-NAME was shown to cause stabilization of mast cells and subsequent neutrophil adhesion (Kubes et al., 1993). Thus, the stimulation of neutrophil infiltration caused by L-NAME may be as a result of mast cell degranulation activated by L-NAME and subsequent induction of neutrophil adhesion and eventually infiltration.

Despite of this discrepancy, our results show that nitric oxide plays an important role in the pathogenesis of allergic rhinitis. Through its powerful vasodilatory effect, nitric oxide may control the filling of nasal capacitance vessels, thus determining nasal patency and mediating acute congestion accompanying allergen challenge.

\subsection*{4.1.4 Effect of heparin on sneezing, nose rubbing, nasal airway pressure and leukocyte infiltration in sensitized ovalbumin challenged guinea pigs}

Heparin is a highly sulfated unbranched glycosaminoglycan. In addition to its well established anticoagulant properties, it exerts other actions including modulation of various proteases (Schwartz et al., 1986), inhibition of cell growth (Castellot et al., 1985), and attenuation of inflammatory responses (Okajima et al., 2001). Heparin is now recognized to interact with a wide range of proteins implicated in inflammatory responses. Many of these heparin-binding proteins are central to the inflammatory
process, including cytokines, growth factors, adhesion molecules, cytotoxic peptides and tissue-destructive enzymes (Rose et al., 2004). Heparin has been proposed to have a regulatory role in limiting inflammation, in part through its capability to bind such proteins, thereby limiting cellular activation and subsequent tissue damage and remodelling (Page 1991). Heparin has particular relevance to allergy and inflammation in that it is found exclusively in mast cells (Esko et al., 2002). Very little research has been undertaken over the past years to study heparin in the treatment of allergic rhinitis.

This is the first study to show the protective effect of heparin in animal models of allergic rhinitis in vivo. Our results show that intravenously administered heparin significantly attenuates the increase in nasal airway pressure after allergen provocation in sensitized guinea pigs. In addition, pretreatment with intravenous heparin significantly reduced leukocyte influx (predominantly eosinophils) 60 minutes after allergen challenge. On the other hand, heparin failed to alleviate sneezing and nose rubbing after allergen challenge in sensitized guinea pigs. Mast cells and eosinophils are crucial for the development of allergic rhinitis. Eosinophils are in fact able to produce a wide array of proinflammatory cytokines and have the capacity to release cytotoxic proteins, including major basic protein, eosinophil-derived neurotoxin, and eosinophil cationic protein, which are potentially harmful for the integrity of the nasal mucosa. In a clinical study, intranasal heparin significantly reduced symptom scores 10 min after antigen challenge. In addition, eosinophil influx in airway mucosa, and the amount of eosinophil cationic protein in nasal lavage fluid were reduced (Vancheri et al., 2001). Moreover, heparin prevents nasal mucosa mast cell degranulation induced by adenosine monophosphate (Zeng et al., 2004).
The mechanisms by which heparin inhibits eosinophil infiltration is not well understood. Heparin, through its capacity to prevent mast cell mediator release (Page 2000), could indirectly produce a downregulation of the expression of a wide range of adhesion molecules and therefore limit eosinophil migration into the nasal mucosa. Furthermore, heparin can also directly regulate cellular diapedesis through the endothelium by reducing the adherence of leukocytes to endothelial cells, probably by increasing the negative charge of the endothelial cell surface (Kanwar et al., 1996). Moreover, other mechanisms have been suggested to explain how heparin can modulate eosinophil recruitment and activation. Heparin can modulate the activity of soluble mediators crucial to the promotion of eosinophil migration. It has been reported that heparin might inactivate platelet activation factor, a cationic protein with a potent chemotactic activity for human eosinophils (Seeds et al., 1993). In this case, the ability of heparin to bind platelet activating factor would result in a reduced cell influx into the tissue. Another study showed that heparin binds to IL-5, the major cytokine regulating eosinophil migration and activation (Lipscombe et al., 1998). In addition, it has been described that there is a possible interaction between eosinophil cationic protein and heparin, suggesting that heparin could neutralize the deleterious effects of eosinophil chemotactic protein (Fredens et al., 1991).

Thus, it could be that heparin ameliorates nasal airway pressure increases by mechanisms involving mast cell stabilization, eosinophil chemotactic protein neutralization, and reduction of eosinophil recruitment. However, although heparin may prevent mast cell degranulation, it failed to attenuate antigen induced sneezing and nose rubbing in sensitized guinea pigs in this study, though heparin attenuates sneezing in
allergic rhinitic patients, suggesting that sneezing and itching mechanisms is more complex in guinea pigs. Taken together, these findings indicate that heparin may have beneficial effects in alleviating symptoms of allergic rhinitis, but more studies are needed to confirm our observations and to define the characteristics of the patients who would benefit most from such a therapeutic approach.

4.1.5 Effect of dexamethasone on sneezing, nose rubbing, nasal airway pressure and leukocyte infiltration in sensitized ovalbumin challenged guinea pigs

Glucocorticoids are used successfully to suppress inflammation in chronic inflammatory diseases, such as asthma and allergic rhinitis (Carreyer et al., 1950; Pipkorn et al., 1987). Corticosteroids act on mast cells, eosinophils, basophils, T-lymphocytes, neutrophils and Langerhans’ cells to alter the release of inflammatory mediators. This includes many of the cytokines that promote inflammation as a result of releasing histamine and attracting cells to infiltrate into affected areas. Corticosteroids also increase the synthesis of lipocortin-1, which has a potent inhibitory effect on phospholipase A2.

Corticosteroids produce pharmacological effects via an initial interaction with a selective cytosolic/nuclear receptor. This receptor, which is located near, or on, the surface of the nuclear membrane in the cytoplasmic space, recognizes the three dimensional characteristics of steroids. If the correct structure is present, the receptor will bind. The receptor-drug complex in dimer form then translocates into the nucleus where it binds via a glucocorticoid responsive element to DNA, and with the involvement of heat-shock proteins regulates the transcription of specific genes to modify the synthesis
rate of individual proteins. Additionally, when present as a monomer drug-receptor complex, the corticosteroids can also act to inhibit gene transcription of inflammatory factors such as many of the cytokines, adhesion molecules and various enzymes (Barnes 1998). Some investigators have suggested that the latter action of corticosteroid-receptor monomers is the most important explanation of their anti-inflammatory actions.

Corticosteroids have been shown to be effective in alleviating chronic symptoms of allergic rhinitis. The role of these agents in acute phase symptoms of rhinitis has not been studies clearly. In this study, we evaluated the effect of corticosteroid dexamethasone on the acute allergic responses in sensitized guinea pigs. Pretreatment with intraperitoneal dexamethasone significantly reduced sneezing frequency but not nose rubbing frequency, evaluated immediately (for 30 minutes) after allergen challenge in sensitized guinea pigs. Intravenously administered dexamethasone failed to ameliorate nasal blockade induced by allergen challenge during the acute phase of rhinitis. In addition, intravenously administered dexamethasone had no apparent effect on the cellular infiltration (no effect on total cell count and eosinophilia measured from nasal lavage fluid) during the acute phase (1hr after allergen provocation) of allergic rhinitis in sensitized guinea pigs.

In terms of sneezing, this is the first study to show that corticosteroids ameliorate sneezing in guinea pig model of allergic rhinitis. However it has been demonstrated in rat model of rhinitis that corticosteroids reduce sneezing responses after allergen challenge (Sugimoto et al., 2000a). The mechanism by which dexamethasone reduces sneezing frequency is not well documented.
Corticosteroids reduce both nasal symptoms and histamine levels of the early phase as well as the late phase reactions when the drug was applied topically in patients with allergic rhinitis (Pipkorn et al., 1987). In addition, it has been demonstrated that topical corticosteroids can reduce inflammation by inhibition of mediators release from mast cells and basophils (Blackwell et al., 1980; Schleimer et al., 1981). Moreover, intranasal fluticasone propionate also reduced both histamine and tryptase in the mucosa of allergic patients (Meltzer et al., 1993). Furthermore, It has been postulated that dexamethasone inhibits histamine H1 receptor upregulation induced by allergen in sensitized rats (Kitamura et al., 2004). Histamine is a main mediator that induces sneezing in allergic rhinitis through its action on H1 receptors on nerve endings. Therefore, dexamethasone reduces sneezing presumably by inhibiting H1 receptor expression on nerve endings and decreasing histamine release from mast cells and eventually lowers sneezing frequency. The effect of corticosteroids on nasal itching has not yet been studied in guinea pig model of rhinitis. Our results show that dexamethasone has no effect on nose rubbing. Since dexamethasone inhibits H1 receptor upregulation in nasal mucosa and it fails to inhibit nose rubbing and mepyramine (refer to antihistamine section) failed to inhibit nose rubbing, it can be concluded that the mechanism of nose rubbing in guinea pig is not simply involve H1 receptor activation. Further studies are needed to explain the mechanisms of corticosteroids in acute nasal responses (sneezing and nasal itching).

Topical nasal corticosteroids are effective in alleviating chronic nasal congestion in patients with allergic rhinitis (Craig et al., 2005; Trangsrud et al., 2002). In our study, intravenous dexamethasone failed to alleviate acute nasal congestion as well as
eosinophil infiltration. In another study, it has been shown that dexamethasone has no inhibitory effect on nasal airway pressure elevation during the early phase but not during the late phase, in a guinea pig model of allergic rhinitis (Yamasaki et al., 2001). Corticosteroids inhibit upregulation of cytokines and adhesion molecules, and suppress the actions of immune cells during an allergic response (Barnes 1998). In chronic phase but not early phase of allergic rhinitis, cytokines and adhesion molecules play major role in induction of nasal congestion. This may explain why dexamethasone failed to inhibit the increase in nasal airway pressure and cellular infiltration in acute phase of rhinitis. Furthermore, it has been demonstrated that allergen exposure may contribute to poor asthma control by reducing glucocorticoid receptor (GCR) binding affinity in mononuclear cells mediated through IL-2 and IL-4 (Nimmagadda et al., 1997). This may indicate that allergen exposure lowers the binding affinity of GCR to corticosteroids and hence reduces their potency.

Despite of their beneficial prophylactic effect in chronic allergic rhinitis, glucocorticoids seems to play minimal role in acute phase responses.
4.2 Conclusions

The method of sensitization used in this study was effective. Sensitized guinea pigs produced acute allergic responses after allergen provocation characterized by sneezing, nose rubbing, increase in nasal airway pressure and eosinophil infiltration. In addition, no effect of intranasal allergen challenge on lung inflation pressure was observed. Furthermore, sensitized guinea pigs produced anaphylactic shock immediately after administration of 1mg/kg ovalbumin intravenously. The response was characterized by rapid increase in blood pressure, heart rate and lung inflation pressure.

Intravenous administration of histamine and acetylcholine produced dose dependent decreases in nasal airway pressure and dose dependent increases in lung inflation pressure. We found that histamine decreases nasal airway pressure by its action on histamine H1 and muscarinic M2 and perhaps M5 receptors, whereas acetylcholine acts on muscarinic M1 receptors to cause decrease in nasal airway pressure. The increase in lung inflation pressure was due to the action of histamine on histamine H1 receptors, and acetylcholine on muscarinic M1 receptors.

Sneezing frequency induced by allergen challenge was attenuated by pre-treatment with mepyramine, cetirizine, and dexamethasone. Nose rubbing frequency provoked by allergen challenge was alleviated by pre-treatment with cetirizine. Increase in nasal airway pressure following allergen provocation was inhibited by pre-treatment with cetirizine, montelukast, heparin and L-NAME. Leukocyte infiltration induced by allergen challenge was prevented by pre-treatment with montelukast and heparin.

In conclusion pathophysiological changes due to allergic rhinitis in guinea pigs resemble to some extent those in humans. The models reported here reflect the
effectiveness of some drugs currently used to treat allergic rhinitis. The models can be used in investigating new potential drugs for the treatment of allergic rhinitis.
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