In vivo studies on the actions of platelet-activating factor and kinins in allergic rhinitis

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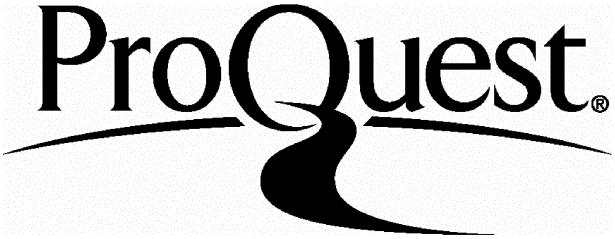

Abstract

Platelet-activating factor (PAF) and kinins have been implicated, among other inflammatory mediators, in the pathophysiology of allergic rhinitis. This thesis describes a human model for quantifying the nasal allergic response, involving rhinomanometry, acoustic rhinometry and nasal lavage techniques, which have been used for examining nasal patency, vascular permeability, mast cell and eosinophil activation. The model was used to investigate the actions of PAF and kinins as putative mediators of allergic rhinitis, in the human nasal airway.

Bradykinin caused dose-related increases in nasal blockage and vascular permeability. A B_1 agonist had no effect in the nasal airway, but the effects of bradykinin could be inhibited by B_2 receptor antagonists. In addition, the effects of bradykinin could also be reduced by prior administration of the H_1 receptor antagonist, cetirizine or terfenadine.

In patients with perennial allergic rhinitis, antigen challenge resulted in an increase in nasal blockage which could be significantly attenuated by a B_2 receptor antagonist, but the antigen had no effect on vascular permeability.

PAF induced a non-specific nasal hyperresponsiveness, which was maximal at 6 hours following treatment. It persisted for up to 24 hours and was associated with eosinophil activation in the nasal cavity. In addition, it was found that the oxygen free-radical scavenger, vitamin E, inhibited hyperresponsiveness and eosinophil activation induced by PAF.

These studies suggest that PAF may be involved in the nasal hyperresponsiveness characteristic of allergic rhinitis and that bradykinin causes vasodilatation and increases in vascular permeability in the nasal airway. Bradykinin appears to have direct B_2 receptor-mediated effects and can possibly also act via a histamine-mediated mechanism. In addition, these studies indicate that the pathophysiology of seasonal and perennial allergic rhinitis may differ.

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Presentations and Publications

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AUSTIN, C.E. & FOREMAN, J.C. (1993). PAF-induced hyperreactivity of the human nasal airway. Br. J. Pharmacol. 108, 78P.

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AUSTIN, C.E., FOREMAN, J.C. & SCADDING, G.K. (1994). [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin is an effective bradykinin antagonist in the human nasal airway. Br. J. Pharmacol. 111, 51P.

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Abbreviations

A min.	Minimum nasal cross-sectional area
BK	Bradykinin
BKA1	[D-Arg^0,Hyp^3,D-Phe^7]-bradykinin
BKA2	[1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin
BKA3	[D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140)
CGRP	Calcitonin gene-related peptide
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
ECP	Eosinophil cationic protein
ELAM	Endothelial leucocyte adhesion molecule
EPX	Eosinophil peroxidase
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GRP	Gastrin-releasing peptide
HDM	House-dust mite, *Dermatophagoides pteronyssinus*
HEPES	4-(2-Hydroxyethyl)-1-piperazine ethanesulphonic acid
5-HT	5-Hydroxytryptamine
ICAM	Intercellular adhesion molecule
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
LT	Leukotriene
Lyso-PAF	1-0-Hexadecyl-sn-glycero-3-phosphocholine.2H_2O
MBP	Major basic protein
MPO	Myeloperoxidase
NAR	Nasal airways resistance
NKA	Neurokinin A
NPY	Neuropeptide Y
Oic	L-[(3aS,7aS)-octahydroindol-2-yl-carbonyl]
OPT	o-Phthalaldehyde

PAF	Platelet-activating factor, 1-0-Hexadecyl-2-0-acetyl-sn-glycero-3-phosphocholine
PG	Prostaglandin
SEM	Standard error of mean
SP	Substance P
Tic	1,2,3,4-Tetrahydroisoquinoline-2-yl-carbonyl
VCAM-1	Vascular adhesion molecule-1
VIP	Vasoactive intestinal peptide

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CHAPTER 1

Introduction

1.1 Introduction

The pathophysiology of the nasal allergic reaction has long been associated with the actions of histamine, but it is now apparent from nasal challenge studies that a wide variety of inflammatory mediators are involved; the relative importance of which remain fully to be elucidated. Histamine challenge may induce some of the signs of acute antigen challenge, but it is clear that the acute nasal response to any single exogenously applied mediator, or even antigen, does not mimic the clinical disease. There appear to be a number of differences, including, the duration of symptoms, different histological profiles and altered responsiveness of the nasal airway. In this context, studies are required further to investigate the mediators and mechanisms which may be responsible for these symptoms.

In recent years, accumulating evidence suggests that both platelet-activating factor (PAF) and kinins may be of importance in the pathophysiology of allergic airway diseases. PAF has been shown in humans, to mimic, to some extent, one of the events underlying allergic asthma, namely a non-specific airway hyperresponsiveness (Cuss *et al.*, 1986). Similarly kinins have been implicated in asthma and allergic rhinitis, since they have the ability to induce basic signs of acute inflammation, both in animals and man (Regoli & Barabe, 1980).

1.2 Allergic rhinitis

Allergic rhinitis describes an inflammatory condition of the nasal mucosa which is characterised by symptoms of nasal congestion, sneezing, rhinorrhoea and pruritus of the nose and eyes. In addition, the nasal airway shows increased responsiveness to topical

stimuli such as histamine and methacholine. Such non-specific hyperresponsiveness is a cardinal sign of allergic rhinitis and has been used to differentiate rhinitics from healthy subjects (Borum, 1979; Druce *et al.*, 1985).

Allergic rhinitis implies an underlying hypersensitivity response mediated by IgE antibodies to foreign allergens. The hallmark of this is the appearance of nasal symptoms following exposure to allergen. Allergic rhinitis may be seasonal or perennial in nature. In London, allergens responsible for seasonal allergic rhinitis include; tree pollens, during April and May; grass pollen in June; moulds, particularly those from *Alternaria alternata* and *Cladosporium herbarum* in August/September; and spores from *Aspergillus fumigatus* in the autumn. Patients with perennial allergic rhinitis usually exhibit positive skin and nasal reactions to the house-dust mite, *Dermatophagoides pteronyssinus*, together with animal danders and certain foods.

1.2.1 Epidemiology and genetic factors

The currently understood epidemiology of allergic rhinitis derives mainly from studies of physician-diagnosed disease. Estimates of seasonal allergic rhinitis vary from 2 to 15% (Malmberg, 1979; Fleming & Crombie, 1987), according the age range examined and the diagnostic criteria. In Britain there has been one community-based survey in which the prevalance of both seasonal and perennial rhinitis was reported (Sibbald & Rink, 1991). This survey involved approximately 3000 adults, aged 16-65 years, registered with a particular London general practice and estimated that 16% of the population had allergic rhinitis; 8% with perennial symptoms alone; 6% with both perennial and seasonal symptoms; and 2% with seasonal symptoms alone. The prevalence of allergic rhinitis appears to be lowest in children under 5 years of age, rising to a peak in young adulthood, and thereafter declining gradually, with the sex ratio among affected individuals being similar (Fleming & Crombie, 1987). The prevalence of atopy was examined in a proportion of these individuals and was found to be highest in those with seasonal allergic rhinitis, 78% as compared with 50% in those individuals with perennial symptoms alone (Sibbald & Rink, 1991). There was also variation reported in symptoms

and their severity between these two types of allergic rhinitis. Subjects with perennial rhinitis had a higher prevalence of nasal blockage, whereas sneezing and itching were the most common symptoms in seasonal allergic rhinitis. It may be that the clinical characteristics of allergic rhinitis are related closely with atopic status, especially in seasonal allergic rhinitis.

Allergic rhinitis generally appears to be associated with asthma and eczema and there is good evidence that all three conditions have some genetic basis. Individuals who form IgE in response to "harmless" environmental factors and display hypersensitivity responses, are called "atopic". Atopy is closely related to serum IgE concentration; the production of which is known to be genetically influenced. The precise genetic control of this is unclear, although likely candidate genes include those for interleukin-4 (IL-4) and interferon-gamma (IFN-γ), since IL-4 is essential for IgE synthesis and IFN-γ has a negative regulatory effect (Plaut *et al.*, 1989). A gene predisposing to atopy has infact been localised on chromosome 11q (Cookson *et al.*, 1989), and more recent evidence shows that the gene for the β subunit of the high-affinitiy receptor for IgE (FcϵRI-β) is closely linked with the gene for atopy on this chromosome (Sandford *et al.*, 1993). The known role of FcϵRI in antigen-induced mast-cell degranulation makes this particularly interesting.

1.3 Pathophysiology of allergic rhinitis

One of the first detailed observations on allergic rhinitis was made in 1873 by Charles Blackley. He noted the nasal response following exposure to allergen; in this case, grass. An immediate effect was recorded consisting of sneezing, hypersecretion and nasal blockage, followed hours later by a recurrence of symptoms, now termed, the late-phase reaction (Blackley, 1873). It is currently appreciated that the major pathological changes which occur in allergic rhinitis are nasal airway inflammation and hyperresponsiveness. Nasal lavage and mucosal biopsy from patients with allergic rhinitis have revealed an increase in inflammatory cells during the pollen season (Pipkorn *et al.*, 1988), and an increase in nasal airway responsiveness has also been demonstrated at this time

(Andersson *et al.*, 1989a).

1.3.1 Nasal airway inflammation

The onset of nasal symptoms is associated with infiltration of the nasal mucosa with inflammatory cells such as mast cells (Viegas *et al.*, 1987), eosinophils (Pipkorn *et al.*, 1988), neutrophils and lymphocytes (Bascom *et al.*, 1988). There are strong correlations between the number cells and the level of the mediator which they release. For example, basophil number correlates with histamine level, as does eosinophil number with the amount of major basic protein (Bascom *et al.*, 1988). This suggests that cellular infiltration is associated with subsequent degranulation of the cells. Thus, increased concentrations of a large number of inflammatory and immunological mediators are found. These include, histamine (Naclerio *et al.*, 1983a; 1983b), bradykinin (Proud *et al.*, 1983) and leukotrienes (Pipkorn *et al.*, 1987a). Inflammation results from the actions of these substances on the nasal vasculature, on the mucus-secreting cells and glands, and on sensory nerves.

Nasal function can be disrupted in three main ways. First, mediators such as histamine, bradykinin and leukotrienes act directly on blood vessels and submucosal glands, causing increased mucosal vascular permeability with oedema, secretion and vasodilatation, leading to nasal blockage. Second, the same mediators will excite sensory nerve endings in the nasal epithelium and deeper mucosal tissues, setting up axon reflexes with the release of neuropeptides, such as, substance P, neurokinin A and CGRP. These neuropeptides will augment vasodilatation and transudation, and may modulate the secretions from submucosal glands (Cauna, 1982). Third, activation of the same sensory nerves also initiates central nervous reflexes via the sympathetic and parasympathetic motor nerves. The responses include sneezing and nasal irritation, reflex nasal vasodilatation and mucus secretion, and actions on the lower airways.

Inflammatory cells and mediators contributing to these responses are discussed in sections 1.4 and 1.5.

1.3.2 Nasal airway hyperresponsiveness

Airway hyperresponsiveness has been stressed over the last decade as one of the major factors involved in the pathogenesis of allergic disease of both the upper and lower airways. Hyperresponsiveness refers to an increased sensitivity of the airways to an allergen or chemical agonist, as indicated by a smaller concentration of agonist needed to initiate a response, as well as a greater maximal response (Woolcock *et al.*, 1984).

In 1960, a link between nasal airway hyperresponsiveness and nasal allergy was identified (Lier & Dishoeck, 1960). This report described patients with grass pollen allergy who demonstrated an increase in nasal responsiveness to intranasally applied veratrine, a mixture of alkaloids. Subsequent studies showed that repeated exposure to ragweed pollen in patients with pollen allergy increased the sensitivity to the pollen (Connell, 1969). Connell referred to this phenomenon as the priming effect and suggested that it was the result of an increased permeability of the mucous membrane, allowing the allergen access more easily to the underlying cells. An association between allergen exposure and nasal hyperresponsiveness has also been demonstrated by Borum and co-workers, where grass pollen allergic patients during the pollen season experienced an increase in nasal responsiveness not only to allergen, but also to other non-specific stimuli such as histamine and methacholine (Borum *et al.*, 1983).

The precise mechanism of non-specific nasal hyperresponsiveness remains to be elucidated. It is not possible to induce hyperresponsiveness to pollen with non-immunological stimuli such as exposure to ammonia (Bacon *et al.*, 1981), or to enhance nasal sensitivity to histamine by repeated challenge with histamine or methacholine (Gronberg *et al.*, 1986). However, allergen challenge does induce an increase in nasal responsiveness to histamine (Andersson *et al.*, 1989b), and methacholine (Klementsson *et al.*, 1990).

Several hypotheses for the mechanism of nasal hyperresponsiveness have been suggested. These include epithelial damage or malfunction, leading to increased access of agent to site of action; increased sensitivity of sensory nerve endings; change in parasympathetic

and/or sympathetic tone on vessels and glands (Andersson & Pipkorn, 1990); upregulation of receptors, e.g. increases in muscarinic receptor number has been found in patients with nasal allergy (Konno *et al.*, 1987); and redistribution towards the mucosal surface or changed numbers of inflammatory cells. The eosinophil in particular has been implicated in the pathogenesis of hyperresponsiveness by virtue of its ability to release mediators which have toxic effects on the epithelium (Frigas & Gleich, 1986). In general, few studies have attempted to correlate mediator levels with non-specific hyperresponsiveness.

In the lower airways, platelet-activating factor (PAF), has been suggested as a mediator responsible for bronchial hyperreactivity (Morley *et al.*, 1985; Benveniste, 1987). PAF is able to induce sustained bronchial hyperresponsiveness in guinea-pigs (Robertson *et al.*, 1988), dogs (Chung *et al.*, 1986), monkeys (Patterson *et al.*, 1984) and man (Cuss *et al.*, 1986).

1.4 Role of inflammatory cells

The initial cellular event in the nasal allergic response is probably the contact of allergen with IgE-bearing mast cells and basophils and possibly other cells on, or in the nasal mucosal surface layer. Such contact and subsequent degranulation leads to the activation of many different cell types which have been shown to have a role in the inflammatory events occuring. Some of these cell types will be described here. However, these cells quite clearly have complex interactions, which in a "cascade" manner, precipitate the allergic response, and determine the degree of inflammation. Such events are further influenced and amplified by other pro-inflammatory mechanisms, such as the action of the adhesion molecules. Nasal biopsy tissue from individuals with perennial rhinitis shows an increased expression, on endothelial cells, of both intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) (Montefort *et al.*, 1992). These adhesion molecules faciltate the infiltration of various cell types into the underlying airway tissue and promote tissue inflammation.

1.4.1 Mast cells and basophils

An association between the mast cell and allergic airway disease has been well documented. Mast cells release a variety of preformed and newly synthesized mediators, which have been shown experimentally to account for several features of allergic rhinitis. These include histamine, PGD_2 and LTC_4, as well as enzymes, such as tryptase, which may degrade certain peptides found in the nasal airway (Caughey, 1989). Heparin is also contained in mast cells bound to histamine. It has been suggested that mast cells may have a possible anti-inflammatory mechanism by virtue of releasing heparin (Page, 1991).

Mast cells have surface receptors which bind IgE with high affinity (FcϵRI). The tail piece or Fc portion of the IgE molecule is bound (at the C_H3 domain) by the high affinity receptors on the mast cell. Cross-linking of IgE leads to mast cell activation and degranulation. Histological evidence suggests mast cell activation and subsequent degranulation occurs in allergic rhinitis (Pipkorn *et al.*, 1988). In addition, nasal biopsy tissue shows an increased number of mast cells in the lamina propria of asymptomatic rhinitis patients (Gomez *et al.*, 1986), and a further increase during the pollen season in patients with hayfever (Viegas *et al.*, 1987). Allergen challenge studies provide evidence for substantial mast cell degranulation occuring in biopsies of nasal mucosa taken 20 minutes after nasal provocation with allergen (Gomez *et al.*, 1986). Further, nasal lavage taken following an allergen challenge, shows increased concentrations of tryptase and histamine (Naclerio *et al.*, 1985). Therefore, mast cell activation appears to be related to the events underlying allergic rhinitis. Sodium cromoglycate and nedocromil sodium which may act by inhibiting mast cell function, (Church & Warner, 1985) are topically effective in reducing nasal symptoms, and also effective in the treatment of ocular allergy.

Several hours after allergen challenge, a further rise in nasal lavage fluid histamine is evident, although there is no increase in PGD_2 (Naclerio *et al.*, 1985). The time at which this occurs corresponds to the late phase reaction. Since histamine is derived from mast cells and basophils, but PGD_2 only from mast cells, it has been suggested that basophils are partly responsible for the late response (Naclerio *et al.*, 1985). The finding that the

late nasal response is characterized by cellular infiltration of, and secretion by basophils and eosinophils (Bascom *et al.*, 1988b) supports the view that the basophil may be important for the late response.

1.4.2 Eosinophils

Tissue eosinophilia is a well established feature of allergic inflammation. Eosinophils have the capacity to generate a number of potent mediators including the basic proteins, eosinophil cationic protein (ECP) and major basic protein (MBP) which are stored in eosinophil granules (Spry, 1985). Other mediators such as PAF (Lee *et al.*, 1984), leukotrienes (Shaw *et al.*, 1985) and reactive oxygen species, including superoxide and hydrogen peroxide (Pincus, 1983) are formed *de novo* after eosinophil activation.

Evidence is accumulating to support a role for eosinophils in allergic rhinitis. Pipkorn *et al.*, (1988) found a seasonal increase of eosinophils in nasal lavage fluid and demonstrated a close correlation between the number of cells, nasal symptoms and pollen counts. There are seasonal changes of eosinophil counts in nasal biopsies taken from patients with hayfever (Lozewicz & Davies, 1990). In addition to eosinophil counts, ECP levels have been measured in nasal lavage. It has been demonstrated that the ECP concentration of nasal lavage is raised 24 hours following nasal allergen challenge in patients with allergic rhinitis (Linder *et al.*, 1987). ECP levels quantitatively correlated with nasal symptoms.

Eosinophil activation involves the release of the toxic basic proteins which have been associated with epithelial damage and airway hyperresponsiveness. Both ECP and MBP cause shedding of guinea-pig isolated tracheal epithelium (Frigas *et al.*, 1980). Epithelial shedding is a characteristic of allergic airway disease and is one of the proposed mechanisms for airway hyperresponsiveness. The release of PAF by eosinophils could contribute to the induction of hyperresponsiveness, since PAF has been implicated as a mediator of bronchial hyperresponsiveness (Cuss *et al.*, 1986). PAF is also a potent eosinophilotactic agent (Henocq & Vargaftig, 1988), and promotes adherence of

eosinophils to endothelial cell monolayers (Kimani *et al.*, 1988), possibly amplifying their effects.

The mechanism of increased eosinophil accumulation, activation and cytotoxicity at the sites of inflammation is largely unexplained. Selective chemotactic factors are thought to contribute to this effect (Wardlaw *et al.*, 1986), but it is also likely that the interleukins, IL-3 and IL-5 are involved. IL-3 and IL-5 prolong the survival of eosinophils and IL-5 has been shown to enhance cytotoxicity and increase respiratory burst activity, promoting the formation of toxic oxygen species (Lopez *et al.*, 1988).

It is, therefore, likely that eosinophils are associated with allergic rhinitis, although evidence is largely circumstantial. Steroids are effective in reducing eosinophil numbers in the nasal mucosa (O'Conner *et al.*, 1991) and this has been attributed to inhibition of the production of the cytokine, IL-5. In this context, it is of interest that allergic inflammation is characterised by the expression of the mRNA for cytokines, IL-3, IL-4, IL-5 and GM-CSF (Kay *et al.*, 1991).

1.4.3 Platelets

Interest in platelets as inflammatory cells has developed in parallel with interest in PAF. Platelets produce PAF, along with leukotrienes and other mediators. In guinea-pig models of asthma, PAF-induced effects are dependent on platelets (Vargaftig *et al.*, 1980), but it is not clear whether the effects of PAF in man involve platelet activation. Platelets can be additionally activated by IgE-dependent mechanisms (Joseph *et al.*, 1983). There are low affinity IgE binding sites (FcϵRII, CD23) on the surface of platelets and IgE-mediated platelet activation probably occurs in allergic airway diseases. Platelet activation markers such as platelet factor 4 and β-thromboglobulin are reported to be elevated in atopic subjects following allergen challenge (Knauer *et al.*, 1981).

1.4.4 Neutrophils

Neutrophils have been identified in nasal lavage in increased numbers following allergen challenge (Bascom *et al.*, 1988a), but their precise role is undetermined. Neutrophils are undoubtedly capable of synthesizing substances which may contribute to the pathophysiology of allergic rhinitis, such as LTB_4, LTC_4 and PAF (Barnes & Costello, 1987). An association between neutrophil infiltration and airway hyperresponsiveness has been suggested in some animal models of asthma (O'Byrne *et al.*, 1987). Neutrophil granules contain a variety of lytic enzymes, such as myeloperoxidase (MPO), which may disrupt epithelial cells. Neutrophils appear in airway tissue along with eosinophils, which have been implicated in epithelial damage (Holtzman *et al.*, 1983; Frigas & Gleich, 1986). However, after challenge with PAF, there appears to be a negative association between the increase in lower airway responsiveness and the number of neutrophils in bronchoalveolar lavage fluid (Wardlaw *et al.*, 1990). Whether this can be extrapolated to the nasal airway is unknown. Nasal allergen challenge studies have shown no relationship between MPO, as a marker of neutrophil activity, and the symptoms of the patients (Linder *et al.*, 1987). To date, there is no convincing evidence that neutrophils play an important role in allergic rhinitis.

1.5 Role of inflammatory mediators

Specific information on the role of a number of mediators involved in generating the symptoms of allergic rhinitis is still evolving. Current knowledge has been obtained from experimental studies which have;

1) demonstrated the local release of mediators in conjunction with an appropriate stimulus;
2) shown that application to the nasal mucosa of the putative mediator induces relevant symptoms;
3) provided evidence that the response to allergen challenge can be blocked pharmacologically with administration locally, or systemically, of a specific antagonist.

Studies involving allergen challenge of nasal tissue *in vitro* (Kainer *et al.*, 1973) and more recently using nasal lavage models for recovery of nasal secretions *in vivo*, have indentified a vast number of putative mediators of inflammation in humans (Naclerio *et al.*, 1983; 1985; Creticos *et al.*, 1984; Pipkorn *et al.*, 1987a; 1987b). The origin of these mediators may be a specific cell population, a group of nerves or plasma (table 1.1).

Putative inflammatory mediators of allergic rhinitis will be discussed with emphasis on platelet-activating factor and kinins.

Cellular origin	Plasma-derived factors	Neural-derived factors
Mast cells/basophils	kinins	substance P
histamine	complement factors	neurokinin A
PGD_2		VIP
tryptase		CGRP
		neuropeptide Y
Eosinophils		
ECP		
MBP		
Neutrophils		
MPO		
Platelets		
PAF		
5-HT		
Various cell types		
PAF		
LTC_4/D_4		
PGE_2		
PGD_2		
PGF_{2a}		

Table 1.1 Origin of putative inflammatory mediators involved in allergic rhinitis

Taken from: Pipkorn, 1989.

1.5.1 Histamine

Histamine has long been recognised as a mediator of the acute allergic reaction. In 1911, Dale demonstrated that histamine had potent vasodilator activity (Dale & Laidlaw, 1911). This, together with its more recently discovered abilities to stimulate sensory nerve endings and induce oedema, as a consequence of opening endothelial cell "tight junctions" in postcapillary venules, has implicated histamine in the pathophysiology of allergic rhinitis (reviewed by Naclerio & Togias, 1991).

Histamine levels in nasal secretions from patients with allergic rhinitis, rise dramatically following nasal allergen challenge, in association with the development of nasal pruritus, sneezing, rhinorrhoea and nasal blockage (Naclerio *et al.*, 1983a; 1983b). The actions of histamine are mediated through three distinct receptors, defined pharmacologically by the actions of their respective agonists and antagonists. Actions of histamine important in allergic rhinitis have been studied *in vivo* by; evaluation of the response to nasal provocation with histamine; and evaluation of the effect of H_1-receptor antagonists on the nasal allergic response. These investigations have revealed that the nasal effects of histamine are primarily mediated via the H_1-receptor (Rokenes *et al.*, 1988; Simons & Simons, 1988). Such effects probably account, almost entirely, for the sneezing and pruritus of allergic rhinitis, and to a lesser extent, for nasal blockage and increases in vascular permeability, since these latter effects are short-lived and of lesser magnitude than those observed with equimolar concentrations of bradykinin (Rajakulasingam *et al.*, 1991) or LTC_4 (Miadonna *et al.*, 1987). H_2-receptor antagonists have a small effect on nasal symptoms in some patients with allergic rhinitis and this is consistent with H_2-receptors, in addition to H_1-receptors, being localised on vascular tissue (Pipkorn, 1990). However, an H_2-receptor antagonist is not sufficient to abolish the residual effects following H_1-antagonist treatment .

Numerous studies with a large range of H_1-receptor antagonists identify benefit in reducing nasal symptoms (Naclerio & Togias, 1991), implying that histamine has a major role in the genesis of the nasal allergic response. A large series of these drugs is available, but the newer, second-generation compounds such as terfenadine, cetirizine,

astemizole and loratadine, are more popular, because they are less lipophilic, reducing their ability to cross the blood-brain barrier, thus reducing their sedative actions. However, the effectiveness of such treatment is probably dependent, to some degree on the allergen inducing the rhinitis. Patients with seasonal allergic rhinitis appear to suffer more from sneezing and itching than nasal blockage, which is the major problem for patients with perennial rhinitis (Sibbald & Rink, 1991). Clinical observations suggest that H_1-antagonists may be more successful in seasonal rhinitics (Dr. G.K. Scadding, Royal National Throat, Nose and Ear Hospital, London - personal communication). Thus, this is consistent with the involvement of other mediators in allergic rhinitis.

1.5.2 Platelet-activating factor

Historical background

The release of histamine from platelets during the acute allergic response in sensitised rabbits was reported in the sixties, and attributed to a factor actively released from leukocytes (Barbaro & Zvaifler, 1966). In 1972, Benveniste and co-workers (Benveniste *et al.*, 1972), demonstrated that this histamine release was the result of IgE-mediated activation of the rabbit basophils, causing the release of a soluble mediator capable of causing platelet activation. Because of this property, the mediator was named platelet activating factor (PAF). These observations raised the question as to whether platelets were involved in allergic reactions.

In 1979, the chemical structure of PAF was identified as 1-o-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (Benveniste *et al.*, 1979; Demopolous *et al.*, 1979; Blank *et al.*, 1979). A number of molecular species belong to this general structure (Pinkard *et al.*, 1984) but those with alkyl groups of C_{16} and C_{18} have been most commonly associated with biological fluids and inflammatory cell types (Ludwig & Pinkard, 1987; Mallet & Cunningham, 1985).

Although studies have been performed with native PAF, the availability of synthetic PAF (Godfroid *et al.*, 1980) has allowed a more extensive investigation of the biological

actions and roles of PAF in physiological and pathophysiological processes.

Biosynthesis and metabolism

PAF is synthesised by two independent enzymatic pathways; the remodeling pathway and the *de novo* pathway (reviewed by Braquet *et al.*, 1987). The remodeling route involves the structural modification of a pre-existing membrane lipid, alkylacyl-glycerylphosphocholine (GPC), via hydrolysis by phospholipase A_2 (PLA_2) to produce lyso-PAF and arachidonic acid. PAF is then formed by a rate limiting step in which a cytosolic acetyl transferase enzyme converts lyso-PAF to PAF. The alternative pathway is the *de novo* synthesis of PAF from alkyllyso-glycerophosphate. This involves a reaction sequence of acetylation and the subsequent transfer of a phosphorylcholine group by a cholinephosphotransferase enzyme to form PAF (Figure 1.1).

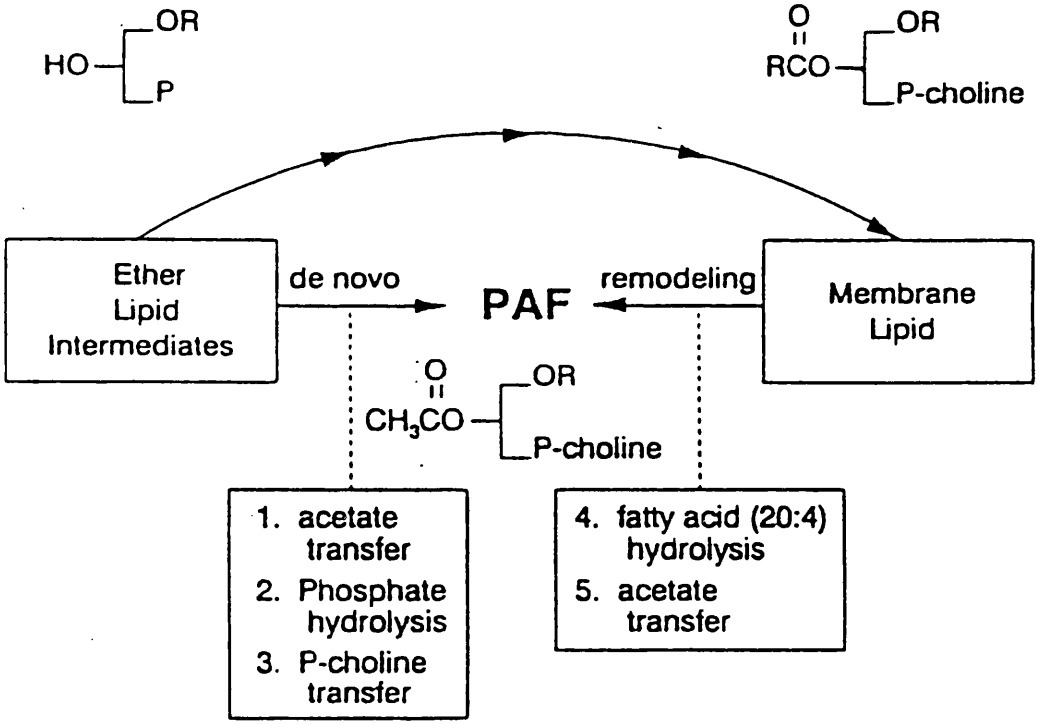


Figure 1.1

Summary of the reaction steps involved in the biosynthesis of PAF by two separate pathways: *de novo* (steps 1 to 3) and remodelling (steps 4 and 5) pathways.

Hypersensitivity and pathophysiological reactions are thought to be caused by activation of the remodeling pathway, whereas the *de novo* route is probably the source of endogenous PAF required for physiological functions (Snyder, 1990). Inactivation of PAF occurs when the acetate group is hydrolysed by one of two types of acetylhydrolase enzyme present in plasma and intracellularly in the cytosol. The product of the action of these enzymes is lyso-PAF: the precursor and the metabolite of PAF.

Cellular origins of PAF

It is now recognised that various cell types are capable of synthesizing PAF in response to a range of allergic and non-allergic stimuli (Braquet *et al.*, 1987). However, measurement of released PAF does not necessarily relate to synthesized PAF, since human neutrophils and moncytes respond to stimulation by synthesizing PAF, but the majority of the lipid remains within the cell (Lynch & Henson, 1986).

A large number of human cell types synthesize and/or release varying amounts of PAF. These include neutrophils, moncytes, alveolar macrophages, eosinophils, platelets, lymphocytes, endothelial cells and fibroblasts (Braquet *et al.*, 1987). It has recently been demonstrated that eosinophils from patients with eosinophilia have an enhanced capacity to release PAF following activation (Lee *et al.*, 1984). It appears that in these individuals, the normally rate-limiting acetyl-transferase enzyme involved in PAF synthesis is no longer rate-limiting (Snyder, 1985). This raises the possibility that allergic individuals may have a genetic defect in this enzyme which may predispose them to abnormal eosinophil activation (Page & Coyle, 1990).

Pharmacological properties of PAF relevant to the pathogenesis of rhinitis

The implication of PAF in the pathogenesis of allergic rhinitis is based on its ability to induce a non-specific bronchial hyperresponsiveness in humans (Cuss *et al.*, 1986). PAF is the only endogenous agent shown to induce bronchial hyperresponsiveness, which can persist for up to 14 days (Barnes, 1986). This correlates well with allergen-induced changes in bronchial responsiveness in asthmatics. In the nasal airway, there is evidence

of PAF metabolic pathway activation following antigen challenge (Miadonna *et al.*, 1989), although, to date, there have been very few studies of the effect of PAF in the nasal airway. Andersson & Pipkorn (1988) reported that nasal instillation of PAF had only a minor effect on changes in nasal patency and nasal symptoms as compared to placebo. This, however, gave no information about a possible action of PAF in causing hyperresponsiveness. These authors then went on to show that a single dose of PAF induced an increase in responsiveness of the nasal vasculature to a subsequent allergen challenge, but, surprislingly, concluded that PAF was not of major importance in the nasal airway. The lack of effect of PAF when applied alone does not mean that it may not have a modulatory role in the nasal airway. In the lower airways, PAF has been shown to induce an increase in airway resistance (Cuss *et al.*, 1986), but a lack of effect in the nasal airway may be attributed to the absence of non-vascular smooth muscle. In addition, Andersson & Pipkorn (1988) used only one dose of PAF and it may be that the dose is critical.

In the lower airways PAF has been shown to elicit increased vascular permeability (Barnes *et al.*, 1986) probably by a direct effect on the vascular endothelial cells since it can only be inhibited by specific PAF-receptor antagonists and not by antagonists of other mediators of vascular permeability, such as eicosanoids and histamine (Archer *et al.*, 1985). PAF has also been demonstrated to induce activation of many cell types (Page, 1988). For example, PAF-induces eosinophil accumulation in the skin of atopic subjects and this is comparable to that induced by allergen (Henocq & Vargaftig, 1986). The possible role of PAF in cell activation and subsequent hyperresponsiveness and inflammation in the nasal airway will be discussed in more detail in chapter 7.

PAF receptors and signal transduction mechanisms

The structural specificity, high biological potency and specific inhibition of PAF by PAF antagonists is indicative of a specific PAF receptor which initiates the responses to PAF *in vitro* and *in vivo*. Radioligand binding studies using [^{3}H]-PAF have provided evidence for the existence of specific PAF binding sites in human lung tissues (Hwang *et al.*, 1985) , although as yet, no similar studies have been performed in human nasal biopsy

tissue. Radioligand binding studies with many other cell types have made it clear that PAF receptors are present in cells responsive to PAF (reviewed by Hwang, 1990). One important outcome of these studies is that there may be heterogeneity in PAF receptors. Ligand binding with structurally different antagonists has identified high- and low-affinity binding sites on cells from the same species (Hwang, 1990). PAF may also act intracellularly and serve as a mesenger within the cell through interaction with intracellular PAF receptors (Shukla, 1992). Whether the PAF receptors on the plasma membrane differ from intracellular receptors is unknown. However, the recent cloning of the PAF receptor from guinea-pig lung (Honda *et al.*, 1991) and new molecular biological approaches may lead to further developments in this area.

PAF antagonists

A number of different classes of drugs have been found to possess antagonistic activity at the PAF receptor. The first selective antagonist described was an analogue of PAF, CV3988 (Terashita *et al.*, 1983). This was followed by a series of compounds structurally similar to PAF with antagonist activity. Other PAF antagonists include those isolated from natural products. Kadsurenone was isolated from the Chinese medicinal herb, *Piper futokadsurae* (Shen *et al.*, 1985) and led to the synthesis of more potent analogues such as L-652-731, (Hwang *et al.*, 1985). Shortly after this, another group of compounds was discovered in the leaves of the tree, *Ginkgo biloba*, the most potent of which is gingkolide B (BN 52021). The gingkolides are an ancient remedy for chest complaints and a combination of them (BN52063), containing 40% gingkolide B, has been demonstrated to be a selective PAF antagonist in healthy volunteers (Chung *et al.*, 1987). Other synthetic molecules, with a diverse range of chemical structures, have also been described as PAF antagonists. These include the triazolobenzodiazepines e.g. alprazolam and triazolam (Kornecki *et al.*, 1985), and their analogues e.g. WEB2086, which are potent PAF antagonists but have no sedative effect (Casals-Stenzel & Weber, 1987), and the pyrrolo-thiazole derivative, RP-48740 (Lefort *et al.*, 1988).

Despite the structural diversity of these compounds, the computerised construction of a

putative PAF receptor indicates that all of the PAF antagonists share spatial features as well as lipophilic and hydrophilic regions that can interact within the constraints of the hypothetical receptor (Braquet & Godfroid, 1987).

1.5.3 Kinins

Kinins are potent vasoactive peptides formed *de novo* in body fluids and tissues. They are derived from the α_2-globulins (high and low molecular weight kininogens) by the action of proteolytic enzymes: tissue and plasma kallikreins. Tissue kallikrein has been reported to be present in both the human nasal airway and human lung, and increased concentrations have been detected following allergen challenge (Baumgarten *et al.*, 1986; Christiansen *et al.*, 1992). This may be of particular relevance to kinin generation during inflammatory reactions in the airways. Two kinins are known to be of physiological relevance in man (Regoli & Barabe, 1980); bradykinin and lys-bradykinin or kallidin.

bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)

lys-bradykinin (Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)
(kallidin)

Once generated, kinins have multiple biological actions and have been implicated in several pathological processes including; asthma, rheumatoid arthritis, shock, pancreatitis, wasp stings (Kellermeyer & Graham, 1968) and more recently, allergic rhinitis (Proud *et al.*, 1983). The actions of kinins are effected through interaction with specific, cell-surface receptors (Regoli & Barabe, 1980).

Virtually all tissues and biological fluids contain specific peptidases (kininases) which can rapidly degrade kinins and thus limit their actions. Kinin degradation is largely initiated by carboxypeptidase N (kininase I) and angiotensin-converting-enzyme, ACE (kininase II). Carboxypeptidase acts via removal of the C-terminal arginine residue, yielding the Des-Arg metabolites, [Des-Arg^9]-bradykinin and [Des-Arg^{10}]-kallidin (Erdos, 1979), while ACE degrades kinins and Des-Arg metabolites by removal of C-terminal di- and

tri-peptides respectively (Sheikh & Kaplan, 1986). In addition, plasma proteases and neutral endopeptidases may also play a role in the metabolism of kinins (Erdos & Skidgel, 1989).

Kinin receptors and antagonists

Kinins act on at least two different membrane kinin receptors, which have been named B_1 and B_2 receptors (Regoli & Barabe, 1980). Kinin receptor classification was originally based on the potency of various agonists in a number of isolated smooth muscle tissue preparations, (Regoli & Barabe, 1980). In the rabbit jugular vein and in canine tracheal strips, both bradykinin and kallidin selectively activate B_2 receptors, while the B_1 agonist, [Des-Arg^9]-bradykinin has no effect (Regoli & Barabe, 1980). However, on the rabbit aorta, [Des-Arg^9]-bradykinin induces contraction, whereas both bradykinin and kallidin have no effect (Regoli *et al.*, 1977). The orders of potency of kinin agonists on the B_1 and B_2 receptors are as follows:

B_1 [Des-Arg^{10}]-kallidin > [Des-Arg^9]-BK = kallidin >> BK

B_2 BK = kallidin >> [Des-Arg^{10}]-kallidin > [Des-Arg^9]-BK

Such orders of agonist potency (Regoli & Barabe, 1980) indicate that most effects of bradykinin *in vivo*, such as inflammation, bronchoconstriction and hypotension, and in isolated tissue preparations (cat ileum, canine tracheal strips, rat vas deferens) are mediated by B_2 receptors. This is supported by the actions of selective kinin receptor antagonists.

Peptide antagonists for the B_1 receptor were developed after the observation that the agonist effect of [Des-Arg^9]-bradykinin was dependent upon the presence of a phenylalanine residue at position 8 in the bradykinin sequence. Following this, further modification in the form of replacement of Phe^8 with aliphatic amino acids resulted in selective competitive antagonist activity (Regoli *et al.*, 1977). One of the first antagonists

produced, [Leu^8]Des-Arg^9-bradykinin and its related analogue, [Leu^9]Des-Arg^{10}-Lys-bradykinin, have been the most potent and widely used B_1 antagonists. [Leu^8]Des-Arg^9-bradykinin inhibits the contractile effects of bradykinin in the rabbit aorta, but not in the rabbit jugular vein, confirming the different kinin receptor types in these tissues.

The availability of specific B_2 receptor antagonists, however, has revealed a complex picture of B_2 receptors. The fundamental work of Vavrek & Stewart (1985) suggested that the structural modification that confers antagonism to bradykinin at B_2 receptors is the replacement of the proline residue at position 7 in the bradykinin sequence with D-phenylalanine. A further selective increase in affinity for the B_2 receptor was achieved by replacing Pro^3 with Hyp and extending the peptide chain at the N-terminal with D-Arg (Regoli *et al.*, 1990). Numerous antagonists have since been developed with additional or alternative structural modifications (Regoli *et al.*, 1990), in the search for a compound with enhanced metabolic stability and increased potency. The availability of these antagonists has revealed possible heterogeneity among B_2 receptors. One of the D-Phe^7 analogues developed, [Thi^{5,8},D-Phe^7]-bradykinin, which was found to be a moderately potent antagonist of bradykinin-induced contraction of the guinea-pig ileum ($pA_2 = 6.4$) (Vavrek & Stewart, 1985) was discovered to have an agonist effect on twitch tension (neurogenic) responses mediated by the pre-synaptic kinin receptor in the vas deferens (Llona *et al.*, 1987), a "classical" B_2 system. However, it acted as an antagonist at the post-junctional receptor site (Llona *et al.*, 1987) which mediates a musculotrophic action. From this study and those of others (Rifo *et al.*, 1987) it has been proposed that possible subtypes of B_2 receptor exist. A B_2 receptor of distinct neuronal location has been identified (Llona *et al.*, 1987) whereas in non-neuronal systems, the presence of at least two further B_2 receptor subtypes have been proposed (Plevin & Owen, 1988).

The existence of an additional novel bradykinin binding site in the large airways has also been suggested. This is based on studies in guinea pig isolated airways, where bradykinin-induced contractions were resistant to inhibition by the B_2 antagonists, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin and [D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, as well as a B_1 antagonist, [Des-Arg^9,Leu^8]-bradykinin, (Farmer *et al.*, 1989). Ligand binding studies indicated specific binding sites for bradykinin but not for [Des-Arg^9]-bradykinin,

ruling out the presence of a B_1 receptor. In addition, neither B_1 nor B_2 antagonists displaced bradykinin from the tracheal binding site, and in lung membranes, the B_2 antagonist, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin displaced only 60% of total specifically bound bradykinin. Thus it was suggested that pulmonary tissue contains a novel, "B_3" receptor (Farmer *et al.*, 1989). Further evidence for the existence of the putative B_3 receptor followed the development of one of the most potent and long-acting bradykinin antagonists to date:
[D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin or Hoe 140 (Hock *et al.*, 1991; Wirth *et al.*, 1991). This compound has increased potency conferred by replacement of D-Phe^7 by D-Tic, and further antagonist potency was achieved by introducing the highly lipohilic, Oic in position 8 (Knolle *et al.*, 1992). This molecule is also highly resistant to enzymatic degradation, since it is not a substrate for ACE or carboxypeptidases (Hock *et al.*, 1991). Studies with this antagonist indicate that it is active at B_2 receptors (Hock *et al.*, 1991; Wirth *et al.*, 1991) and also in tissues where the effects of bradykinin are resistant to other B_2 antagonists. These tissues include the guinea-pig trachea (Perkins *et al.*, 1991) and taenia caeci (Field *et al.*, 1992), both of which are proposed to contain the putative B_3 receptor (Farmer *et al.*, 1989; Perkins *et al.*, 1991; Field *et al.*, 1992).

Further improvements in the selectivity of kinin antagonists are needed to identify possible kinin receptor subtypes. Many of the B_2 antagonists available are partial agonists (Rifo *et al.*, 1987) and release other mediators such as histamine (Devillier *et al.*, 1988); some have low affinity and are not completely selective for B_2 receptors, having some affinity at B_1 sites (Regoli *et al.*, 1986). At present, the lack of inhibitory effect of B_1 and B_2 receptor antagonists on bradykinin-induced contractions in the guinea-pig airways, could be explained by a non-receptor action involving the activation of the arachidonic acid cascade (Rhaleb *et al.*, 1988).

Characterisation of kinin receptors may proceed in the future by applying new molecular biological approaches. A human kinin B_2 receptor has recently been cloned from a human lung fibroblast cell line (Hess *et al.*, 1992), although the agonist and antagonist properties of bradykinin analogues at the cloned receptor do not match strictly the pharmacological profile described for the rat or guinea-pig B_2 receptor subtypes or the putative B_3 type.

This discrepancy has been attributed to species differences but may also imply that the existence of B_3 receptors and B_2 subtypes is questionable.

To date, bradykinin receptor subclassification has been based on functional and ligand binding studies. Signal transduction pathways should also be considered, but at the present time, this is complicated by the fact that B_1 and B_2 receptor-mediated responses are thought to share the same transduction mechanism (Farmer & Burch, 1991a). Activation of bradykinin receptors leads to stimulation of the activity of phosphatidylinositol-specific phospholipase C, resulting in the formation of inositol phosphates and diacylglycerol (Burch *et al.*, 1990). In most tissues, eicosanoid synthesis is enhanced, probably by release of arachidonic acid from diacylglycerol produced by inositol lipid hydrolysis (Farmer & Burch, 1991a). Both bradykinin receptor- mediated inositol lipid hydrolysis and eicosanoid release are dependent on the coupling of the receptor to one or more guanine nucleotide binding proteins, - G proteins (Francel *et al.*, 1989), suggesting that bradykinin receptors belong to the class of receptor comprising seven hydrophobic transmembrane spanning regions, like muscarinic receptors, adrenoceptors and a variety of peptide receptors. In addition, bradykinin receptor activation often leads to increased cellular accumulation of cyclic AMP. This may be due to stimulation of the biosynthesis of eicosanoids which bind to their receptors and activate adenylate cyclase (Brunton *et al.*, 1976).

Kinins in inflammation

Evidence is accumulating to suggest that kinins have a central role in the pathophysiology of inflammation, such as that associated with asthma and rhinitis. The basis of this is that kinins have actions which reproduce the symptoms of acute inflammation, including vasodilatation, increased vascular permeability and stimulation of sensory nerves (Regoli & Barabe, 1980). Kinin B_2 receptors have been mapped out at potential sites of inflammation in human and guinea-pig lung by autoradiography with [^{3}H]-bradykinin (Mak & Barnes, 1991). Binding sites were revealed in endothelial cells of bronchial and pulmonary vessels, epithelial cells, airway smooth muscle, submucosal glands and nerve

endings. The autoradiographic distribution of B_2 receptors in the human nose has also been investigated. Binding with [^{125}I]-bradykinin to human inferior turbinate nasal mucosa was examined. This revealed binding sites on small arteries, venous sinusoids and submucosal fibres, but no specific binding to mucosal glands or goblet cells was found (Baraniuk *et al.*, 1990c). The binding of bradykinin to vessels rather than glands is consistent with a direct effect of bradykinin in altering vascular tone and permeability in the human nasal mucosa (see chapter 4). In addition to kinin receptors being localised at sites where inflammation in the airways is known to occur, kinins are also found in increased amounts at the site of tissue injury. Furthermore, local conditions related to inflammation, such as increased pH of plasma exudate, favour the accumulation of kinins (Polosa, 1993). There are also reports suggesting that the kinin-kallikrein pathway is activated in inflammation. A number of inflammatory cell types liberate proteases which may have kallikrein-like activity. Both mast cell (Proud *et al.*, 1985) and basophil (Newball *et al.*, 1979) proteases display kallikrein activity *in vitro*. Therefore, it is possible that this activation pathway may be important *in vivo*, leading to enhanced kinin generation at multiple sites.

The function of kinins in allergic rhinitis will be addressed in chapters 4 and 5.

1.5.4 Eicosanoids

Leukotrienes

In vitro stimulation of human eosinophils, mast cells, monocytes, and basophils leads to the generation of either the sulphidopeptide LT's (LTC_4, LTD_4 and LTE_4) or LTB_4, through the metabolism of membrane-bound arachidonic acid by the enzyme 5-lipoxygenase (Lewis *et al.*, 1990). These inflammatory mediators can increase vascular permeability at the site of post-capillary venules and facilitate plasma leakage and oedema, as well as cellular diapedesis (Drazen *et al.*, 1980). LTB_4 is a potent chemoattractant for neutrophils (Palmblad *et al.*, 1981), and both the sulphidopeptide LT's and LTB_4 enhance human airway mucus secretion, with LTD_4 being the most potent stimulant studied (Marom *et al.*, 1982). This knowledge encouraged a number of studies

by various investigators to determine the participation of the LT's in the events underlying rhinitis.

Initial studies correlated antigen challenge with increased levels of sulphidopeptide LT's in subsequent lavage (Creticos *et al.*, 1984). This applied to allergic subjects only, and moreover, was specific to the allergic reaction, since nasal methacholine challenge had no effect on LT detected in nasal secretions (Shaw *et al.*, 1985). Analysis of lavage fluid obtained after antigen challenge demonstrated that the sulphidopeptide LT's, LTC_4, LTD_4 and LTE_4 were all present, immediately and several hours after challenge, although LTC_4 predominated in both the early and late phase responses (Pipkorn *et al.*, 1987a). Small amounts of LTB_4 have been identified in lavage fluid samples taken during the early response to antigen challenge (Shaw *et al.*, 1985; Freeland *et al.*, 1989) and also during the late response, although this had no relation to neutrophil counts, a likely source of LTB_4 (Freeland *et al.*, 1989). Other studies have attempted to correlate seasonal exposure to antigen in grass-sensitive individuals with LT levels by examining urinary levels of LTE_4. However, this study failed to detect any significant changes between LTE_4 levels in and out of pollen season (Taylor *et al.*, 1989).

Nasal provocation tests with LTD_4 have resulted in significant increases in nasal airway resistance and nasal blood flow, but no effect was observed on nasal secretions (Bisgaard *et al.*, 1986). Okuda *et al.*, (1988) performed studies which support these observations, and likened the LTD_4-induced increase in nasal airway resistance to that observed after antigen challenge. Thus, these investigators proposed that LTD_4 is primarily involved with the nasal congestion associated with allergic rhinitis. It should be noted, however, that this may not be an exclusive effect of LTD_4. On the basis of the study by Pipkorn *et al.*, (1987a), LTC_4 may induce nasal symptoms in provocation studies, but has not been employed, because it is rapidly metabolised.

Leukotriene antagonists have been developed, but have not yielded much information regarding the role of LT's in rhinitis because of their low potency. In one study, the orally administered LTD_4 antagonist, L-649,923 had no effect when compared with placebo on the response to nasal antigen challenge (Flowers *et al.*, 1990). This was

attributed to low affinity of the compound for the receptor, but may also indicate that LT's other than LTD_4 are more important in rhinitis. In another study, Knapp (1990) showed that the 5-lipoxygenase inhibitor, A-64077 had some effect in reducing allergen-induced nasal congestion and LTB_4 production. Considering these studies, a possible role for the LT's in allergic rhinitis is possible, although further investigation with more potent antagonists, to determine the relative importance of the LT's, is required.

Prostaglandins

Prostaglandin (PG) D_2 has been found in increased concentrations in the nasal lavage of subjects immediately following antigen challenge (Naclerio *et al.*, 1983a; 1983b). However, the functional significance of this is unclear. PGE_2 is known to produce vasodilation of nasal resistance vessels (Lozewicz & Davies, 1990), but the relative contribution of this to nasal blockage occuring in allergic rhinitis compared with mediators such as bradykinin is unknown. It has been suggested that prostaglandins may indirectly cause the effects of other mediators in the nasal airway; namely, bradykinin. However, treatment of subjects with aspirin prior to bradykinin challenge, had no effect on bradykinin-induced increases in vascular permeability or symptom scores for nasal blockage (Churchill *et al.*, 1991). Similarly, cyclooxygenase inhibition in patients with allergic rhinitis does not appear to have a beneficial effect (O'Byrne & Fuller, 1989).

1.5.5 Neuropeptides

Neuropeptides are located in all 3 types of nerve fibres innervating the nose; type C sensory nerves, parasympathetic and sympathetic nerves. Sensory fibres from the trigeminal nerve contain several neurotransmitters including calcitonin gene-related peptide (CGRP), the tachykinins, substance P (SP) and neurokinin A (NKA) (Lundberg *et al.*, 1987), and gastrin-releasing peptide (GRP) (Sunday *et al.*, 1988). Parasympathetic neurones contain, in addition to acetylcholine, vasoactive intestinal peptide (VIP) and peptide histidine methionine (Said & Mutt, 1988) and sympathetic neurones contain noradrenaline and neuropeptide Y (NPY) (Baraniuk *et al.*, 1990a).

Neuropeptides produce many different effects mediated by surface receptors (reviewed by Barnes *et al.*, 1991a; 1991b). Functional responses in the nasal airway and their relevance to allergic rhinitis continue to be investigated. Immunohistochemistry and autoradiography has been used to locate neuropeptides within the nasal mucosa and to identify the distribution of specific neuropeptide binding sites. These investigations, together with results from studies using nasal turbinate explants to assess the effect of neuropeptides on glandular secretion (Raphael *et al.*, 1991), have indicated the function of each peptide. Submucosal glands are rich in both receptors and fibres for, in descending order, GRP, VIP, SP and NKA. These have been shown to be secretagogues, with an order of potency corresponding to their receptor density (Raphael *et al.*, 1991). The pro-secretory activity of VIP is evident from therapeutic interventions with cholinergic antagonists. VIP is a product of parasympathetic neurones and therefore can be inhibited by atropine-like drugs, such as ipratropium bromide, a derivative of isoproyl noratropine, which has a low lipid solubility and a low level of systemic side effects. This compound has an anti-secretory action widely demonstrated in rhinitic subjects (Dolovich *et al.*, 1987). CGRP and NPY receptors are located on arterioles. CGRP has been shown to cause vasodilatation (Baraniuk *et al.*, 1990b), consistent with its potent vasodilatory action in skin (Brain *et al.*, 1985; Piotrowski & Foreman, 1986), thus contributing to nasal congestion. NPY is a potent vasoconstrictor, which reduces blood flow and microvascular leakage, through the reduction in the perfusion of permeable postcapillary venules (Baraniuk *et al.*, 1990a). Such a vasoconstrictor action can be mimicked by the use of α-adrenoceptor agonists, leading to relief of nasal blockage. Pseudoephedrine hydrochloride and phenylpropanolamine hydrochloride are the most commonly used α-agonists or "decongestants".

The recent development of specific receptor antagonists for the tachykinin, NK_1 and NK_2 receptors, which are activated preferentially by SP and NKA respectively (Barnes *et al.*, 1991a), is allowing further elucidation of the role of tachykinins in airway function. For example, the NK_1 receptor antagonist, CP-96345 reduces plasma extravasation evoked by bradykinin in guinea-pig trachea (Geppetti *et al.*, 1993), whereas the NK_2 antagonist, SR-48968 had no effect on bradykinin effects in a similar model (Sakamoto *et al.*, 1993). As yet, there have been no studies with such compounds in the nasal airway.

1.5.6 Oxygen-derived free radicals

Oxygen-derived free radicals (superoxide anions, O_2^- and hydroxyl radicals, ·OH), or metabolites (hydrogen peroxide, H_2O_2 and hydrochlorous acid, HOCl) are produced by several inflammatory cell types and can exert a range of cytotoxic effects. Reactive oxygen species have been implicated in airway hyperresponsiveness *in vitro* (Webber *et al.*, 1992) and in animal models (Murlas & Roum, 1985; Katsumata *et al.*, 1990). In addition to inflammatory cell types, it has been shown that superoxide anion generation can be stimulated by platelet-activating factor (Kato *et al.*, 1993). Such PAF-induced enhancement of O_2^- production can be blocked with a specific PAF receptor antagonist (Kato *et al.*, 1993). This is of particular interest considering that platelet-activating factor induces a non-specific hyperresponsivenes in the human bronchial airway (Cuss *et al.*, 1986). The role of reactive oxygen species in nasal hyperresponsiveness is discussed in chapter 7.

1.5.7 Cytokines

Local release of cytokines in the nasal mucosa from immunologically stimulated cells contribute to a wide range of cellular effects. Factors have been isolated from nasal biopsies of rhinitic patients that stimulate the formation of eosinophils from precursor cells in the circulation, and these have been identified as locally generated cytokines (Ohnishi *et al.*, 1988).

In vitro studies show that interleukin (IL)-3, IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF) are involved in eosinophil recruitment and appear to augment eosinophil mediator release (Wardlaw *et al.*, 1986; Kroegel *et al.*, 1989). IL-4 may be important in the regulation of IgE production from B-lymphocytes since a monoclonal antibody to IL-4 inhibits IgE production, with no effect on IgG production (Finkelman *et al.*, 1986). IL-1 and tumour necrosis factor (TNFα) have been shown to increase the expression of intercellular adhesion molecule-1 (ICAM-1) in various cell types (Rothlein *et al.*, 1988). Adhesion molecules are important in directing the adhesion of leukocytes to blood vessels at the sites of inflammation. Thus, in this way, IL-1 has

potential for modifying the inflammatory response in the nasal mucosa. More recently, it has been shown that IL-1 can modulate the responsiveness to bradykinin in human synovial cells by upregulation of kinin receptor expression (Bathon *et al.*, 1992). This raises the question of a modulatory role of this cytokine in the responsiveness to bradykinin in the human nasal airway, since subjects with chronic rhinitis show strikingly enhanced responses to bradykinin when compared with normal subjects (Dr D. Proud, Johns Hopkins University School of Medicine, Baltimore, USA - personal communication).

Unfortunately, there are no specific antagonists for cytokine receptors, but studies with monoclonal antibodies directed at specific cytokines may reveal information on their actions in the nasal inflammatory response.

1.6 Aims of project

The aims of the following investigation were:

- To develop a human model in which specific features of the nasal allergic response could be studied, including nasal airway patency, vascular permeability of the nasal mucosa, mast cell and eosinophil activation.

- To examine the effects of platelet-activating factor in the nasal airway, with respect to hyperresponsiveness.

- To explore the effects of kinins in the nasal airway and the types of kinin receptor mediating changes in nasal function in human subjects by utilising selective kinin agonist and antagonist peptides.

- To determine the role of kinins in the nasal response to antigen challenge in patients with allergic rhinitis.

- To develop an animal model in which the actions of putative mediators of allergic rhinitis may be studied.

CHAPTER 2

Materials and methods

2.1 Materials

The materials used in this study and their source are listed below.

MATERIAL	SOURCE
Bradykinin (BK)	Peninsula Labs., St.Helens, U.K.
[D-Arg^0,Hyp^3,D-Phe^7]-BK (BKA1)	Peninsula Labs., St.Helens, U.K.
[1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-BK (BKA2)	Peninsula Labs., St.Helens, U.K.
[D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (BKA3)	Bachem, Saffron Walden, U.K.
Cetirizine	UCB Pharma Ltd., Belgium.
Dermatophagoides pteronyssinus	Allerayde, Nottingham, U.K.
[Des-Arg^{10}]-kallidin	Peninsula Labs., St.Helens, U.K.
ECP RIA kit	Pharmacia, Uppsala, Sweden.
Evans blue	Sigma Chemical Co., Poole, U.K.
Heparin	CP Pharmaceuticals Ltd., Wrexham
Histamine diphosphate	Sigma Chemical Co. , Poole, U.K.
Histamine RIA kit	Immunotech Int., Marseille, France
Albumin immunodiffusion plates	Behring, Milton Keynes, U.K.
Lyso-PAF (C_{16})	Novabiochem, Nottingham, U.K.
o-pthalaldehyde	Sigma Chemical Co., Poole, U.K.
PAF (C_{16})	Novabiochem, Nottingham, U.K.
Protein standard serum	Behring, Milton Keynes, U.K.

Terfenadine	Marion Merrell Dow Ltd., Uxbridge
Urethane	Sigma Chemical Co., Poole, U.K.
Vitamin E	Pharmadas Ltd., Greenford, U.K.

All other substances were of Analar or similar quality.

2.1.2 Dilution of nasal challenge agents

Histamine as the diphosphate salt was dissolved in saline (NaCl, 154 mM) to make solutions ranging from 1 mg/ml to 10 mg/ml. Platelet-activating factor (PAF) and lyso-PAF were dispersed in distilled water to make a suspension of 0.6 mg/ml. Bradykinin, [D-Arg^0,Hyp^3,D-Phe^7]-BK, [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK and [Des-Arg^{10}]-kallidin were dissolved in saline to achieve concentrations from 0.1mg/ml to 10mg/ml, depending on the dose required. [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-BK was dissolved in glacial acetic acid, neutralised with sodium hydroxide and made up to a 1mg/ml solution with distilled water.

D. *pteronyssinus* was dissolved in saline to make a stock concentration of 100,000 units/ml, where one unit (U) is an arbitrary measure related to the biological potency determined by skin prick test against histamine dihydrochloride, 1mg/ml. Sterile saline and sterile distilled water were used throughout, as indicated, for making up stock solutions and their subsequent dilution.

2.2 Methods for human nasal provocation studies

Nasal provocation model

A universal experimental model of rhinitis has yet to be found, because of the diverse stimuli which can provoke the disorder and the variable clinical features. However, many systems have been developed in an attempt to study rhinitis. These include exposing subjects to natural allergens during the appropriate season (Pipkorn *et al.*, 1988), and performing nasal provocation tests in the laboratory with allergens (Naclerio *et al.*,

1983a), viruses (Gaffey *et al.*, 1987), mediators (Raphael *et al.*, 1988), and environmental stimuli (Anderson *et al.*, 1972). Such nasal provocation allows for a controlled assessment of the nasal response between individuals. Methods used to quantify nasal responses include, recording symptoms scores (Rajakulasingham *et al.*, 1991), counting sneezes or number of tissues used (Borum, 1979), measuring nasal airways resistance (Corrado *et al.*, 1986), measuring volume of nasal secretions (Borum, 1978), and analysing nasal secretions for mediators (Togias *et al.*, 1985), cellular constituents (Bascom *et al.*, 1988a) and protein content (Raphael *et al.*, 1988). Experimentally, these systems allow the study of a particular mediator and also allow the investigation of the effects of various pharmacological agents and allergens.

In this project, a human model was developed and used to study pharmacologically-induced changes in nasal function. This consisted of nasal challenge with pharmacological agents or allergen, by aerosol, and subsequent quantitative measurements consisting of analysis of mediators and albumin in nasal lavage, measurement of nasal airway resistance by posterior rhinomanometry, measurement of nasal volume and cross-sectional area by acoustic rhinometry and recording of symptom scores.

2.2.1 Subjects

For all studies except those with atopic patients, normal, healthy volunteers with age range 21 to 42 years were used. Subjects suffering from upper respiratory tract infection or reporting any nasal symptoms were excluded. Subjects were taking no medication at the time of, or in the two weeks prior to the experiments. All subjects gave informed consent and the study was approved by the local Ethics Committee at University College London. Experiments were performed in a laboratory with controlled temperature and humidity.

For the study involving atopic patients, selection was made on the basis of a history of rhinitis, a positive skin prick test and a positive nasal challenge to the house-dust mite, D. *pteronyssinus*. Patients taking part were within the age range, 18 to 56 years and had

anatomically normal nasal cavities, no evidence of nasal polyposis or upper respiratory tract infection and had been off all oral and intranasal therapy for at least 4 weeks prior to the study. Patients with asthma were excluded. All patients gave informed consent and the study was approved by the local Ethics Committee at the Royal National Throat, Nose and Ear Hospital, London.

2.2.2 Rhinomanometry

Rhinomanometry is the measurement of transnasal pressure and nasal airflow and the subsequent calculation of nasal resistance. The basic method involves a pressure transducer and a flow meter connected to a microcomputer for data analysis. Active anterior rhinomanometry involves measuring the pressure and flow in each nostril separately, via a nasal pressure sensing tube, then calculating resistance; whereas active posterior rhinomanometry involves measuring both nasal passages simultaneously, using an oral pressure cannula and provides a value of total nasal airway resistance. Active posterior rhinomanometry has the advantage of eliminating bias in the data resulting from nasal cycling.

Active posterior rhinomanometry, employing a Mercury Electronics (Glasgow, U.K.) NR6D rhinomanometer, was used in these studies to obtain measurements of nasal airway resistance (NAR). The instrument was programmed to calculate the resistance to airflow at a reference pressure of 75Pa. The subject held a mask, with airtight seal over the nose and mouth, and breathed through the nose. Airflow was monitored by a pneumotachograph and pressure within the oropharynx was monitored by a pressure transducer connected to a cannula placed over the tongue and held through sealed lips. A visual display of the pressure/flow relationship provided by the computer was used to detect any abnormalities in measurement. Abnormal pressure/flow curves may be caused by soft palate and tongue movements, preventing a continuity between the oral cavity and the pharynx. Such problems are easily identifiable and subjects were trained to eliminate them. The instrument was programmed to give the mean NAR over four breath cycles, and for each subject, three consecutive series of four breath cycles were used to calculate

an overall mean NAR from twelve breaths. Readings were only accepted if the coefficient of variation of the overall mean NAR for a subject was less than 20%.

2.2.3 Acoustic rhinometry

Measurements of nasal cross-sectional area and volume were made by acoustic rhinometry using equipment supplied by 'gm' Instruments (Kilwinning, U.K.). The apparatus consists of a spark generator that produces a sound impulse, a wave tube through which the acoustic pulse is transmitted to the anterior nares of the subject, a microphone with amplifier for measurement of sound, and a computer for data acquisition and analysis. The spark generated is discharged between two electrodes placed in the end of the wave tube and an audible sound impulse is created. The acoustic pulse propagates down the wave tube, passes the microphone and enters the nasal cavity via a 6cm long plastic tube inserted into the wave tube. The free end of the tube was inserted a few millimetres into the anterior nares. Three sizes of tubes were available to ensure a tight-fit in the nostril of each subject. The sound waves reflected from the nasal structures pass back down the wave tube and are received by the microphone and analysed by the computer. The data obtained is presented as an area-distance function and provided measurements of volume at various specified areas in nasal cavity and a minimum nasal cross-sectional area (A min.). The measurement lasts less than 10ms.

2.2.4 Nasal Lavage

Nasal lavage was used to provide a source of upper respiratory tract cells and chemical mediators. Nasal lavage involves the instillation of a liquid into the nasal cavity and the subsequent recovery of that liquid admixed with a sample of nasal secretions. Preliminary experiments were performed to identify the most suitable method from those described in the literature. These are outlined below:

1) Head extended 45° from the horizontal with nasopharynx occluded voluntarily by

the soft palate. 5ml warmed saline introduced into each nostril separately and expelled into a tube after 10 seconds hold (Naclerio *et al.*, 1983b).

2) Instillation of 100μl portions of saline by a nasal spray. The subject then expels the fluid onto a plastic dish, (Linder *et al.*, 1988).

3) Catheter placed along the floor of the nose, inserted to 4cm. Saline administered from a nebulizer bottle; suction applied at 200mmHg and secretions removed from a specimen trap and transferred to a test tube, (Druce *et al.*, 1985).

4) A Foley urinary catheter, modified by cutting off the tip distal to the balloon, is inserted into the nasal vestibule and the balloon inflated to provide a seal. The head is dorsiflexed to 45° and 8ml saline is instilled through the catheter using a bladder syringe and irrigated once. It is then aspirated, and the balloon deflated and removed, (Hilding, 1972).

The method of Naclerio *et al.*, (1983b) was selected for reasons of ease, relatively uniform recovery of approximately 80% of the instilled volume, and good tolerance among the subjects. Naclerio and co-workers (1983b) report that volunteers have tolerated lavages as frequently as every 2 minutes, and at 4hr intervals for as long as 6 days. The technique therefore permits sampling before and after natural or induced provocation and was performed as follows:

Subjects were in a sitting position with the head extended 30° from the horizontal. Warmed sterile saline (5 ml) was instilled into a nostril while the subject held the tongue against the soft palate and abstained from breathing or swallowing. After 10 s the subject leaned forward and expelled the lavage fluid into a collection vessel, which was stored on ice until the completion of the experiment. Approximately 4 ml of the wash was recovered on each occasion. In each experiment, three initial washes were collected in order to remove pre-existing nasal secretions and the third of these was retained and served as a baseline. The lavage fluid was centrifuged at 4°C for 10 min at 1000 g, after

which the supernatants were separated, and stored at -20°C until analysis.

2.2.5 Symptom scores

A subjective measure of the effects of each nasal provocation was made by recording symptom scores. Subjects were asked to score values for nasal blockage, itching, sneezing and running on 10cm visual analogue scales (Aitken, 1969). Symptoms were scored at the start of the experiment, after vehicle (saline) challenge and after every subsequent challenge.

2.2.6 Nasal challenge

Methods described for introducing challenge agents into the nose have included; aerosol administration, application of paper strips soaked in substance applied to the inferior turbinate, and administration by tuberculin syringe on to the inferior turbinate.

Nasal challenge using a nasal pump spray (Perfect-Valois, UK Ltd.) was chosen for this study because it is more likely that substance will be delivered throughout the nasal cavity, and be more reliably and reproducibly delivered. The spray delivers 100μl per activation with a 98% degree of accuracy, and so the dose is controlled by the concentration of the solution. The device was placed in one nostril and activated once, then repeated for the opposite nostril. All solutions were at room temperature, and fresh solutions of all challenge agents were made each day from stock solutions stored at -20°C.

2.2.7 Basic protocol

Detailed study protocols are presented together with the data in each chapter. The following however, is common to all studies:

At each visit, subjects were first challenged with vehicle, then measurements of NAR by active posterior rhinomanometry, or measurements of A min. by acoustic rhinometry were taken every 5 minutes over a fifteen minute period to provide a baseline. The mean of these data was calculated and this was used as a baseline measurement. Following every challenge, measurements were made at 2, 5, 10, 15 and 20 minutes. At least 30 minutes was allowed between challenges.

In the case of studies involving nasal lavage, subjects underwent three lavages initially to remove pre-existing nasal secretions, followed by challenge with vehicle and a lavage 10 minutes later. The concentration of the substance in question (e.g. albumin, histamine, ECP, depending on the experiment) measured in the post-vehicle sample, formed the baseline. Nasal lavages were performed after every subsequent drug/allergen challenge.

2.2.8 Data analysis

Data analysis and statistical evaluation will be dealt with in each chapter individually. However basic data handling procedures are explained below.

Both changes in NAR and A min. in response to each challenge were presented as the percentage change from the values obtained after vehicle challenge. This normalised the data, accounting for individual anatomical differences in the nasal airway which give rise to variable baseline measurements. Measurements of A min. were expressed as the mean value of both nostrils, to make them comparable with NAR measurements and to avoid misinterpretation of unilateral changes due to the nasal cycle. The response to each challenge was quantified as the maximal percentage change in NAR or A min. for each dose during the post-challenge period.

The total symptom score at each stage was obtained by measuring the lines on the visual analogue scales and adding them. The difference between the mean score after each challenge and the mean score after vehicle challenge was estimated as a percentage of the vehicle score.

All data was expressed as mean ± s.e.m. Data was only accepted if the coefficient of variation (CV) for the values used to calculate the mean was less than 20%. Student's t-test was used for the evaluation of the difference between control values and those obtained after challenge. Both unpaired and paired tests were used as appropriate. Repeated measures analysis of variance (one-way ANOVA) was used to evaluate the effect of increasing doses of challenge agent on the nasal parameters measured. A probability value, p, of less than 0.05 was considered significant.

2.3 Biochemical analyses

2.3.1 Albumin measurement

Albumin was measured by a radial immunodiffusion assay (Mancini *et al.*, 1965). A protein standard serum was used to establish a calibration curve, and human sera of known albumin concentration were used as controls to confirm the function of the immunodiffusion plates. The plates were loaded with 20μl of standard or sample into each well and left to stand open for 30 minutes, until the applied fluid had diffused into the agarose gel. The plate was then closed and incubated for 48 hours at room temperature.

A standard curve was constructed by measuring the diameter in mm of the precipitate rings formed around the wells containing the standard solutions and calculating the squares of the ring diameters. These area measurements (mm^2) were plotted as a function of the concentration of albumin in the standards (mg/dl). The concentration of albumin in the samples was read directly off the graph. The assay range was 2.5 - 44.0 mg/dl (Behring Diagnostics). Figure 2.1 is an example of a standard curve.

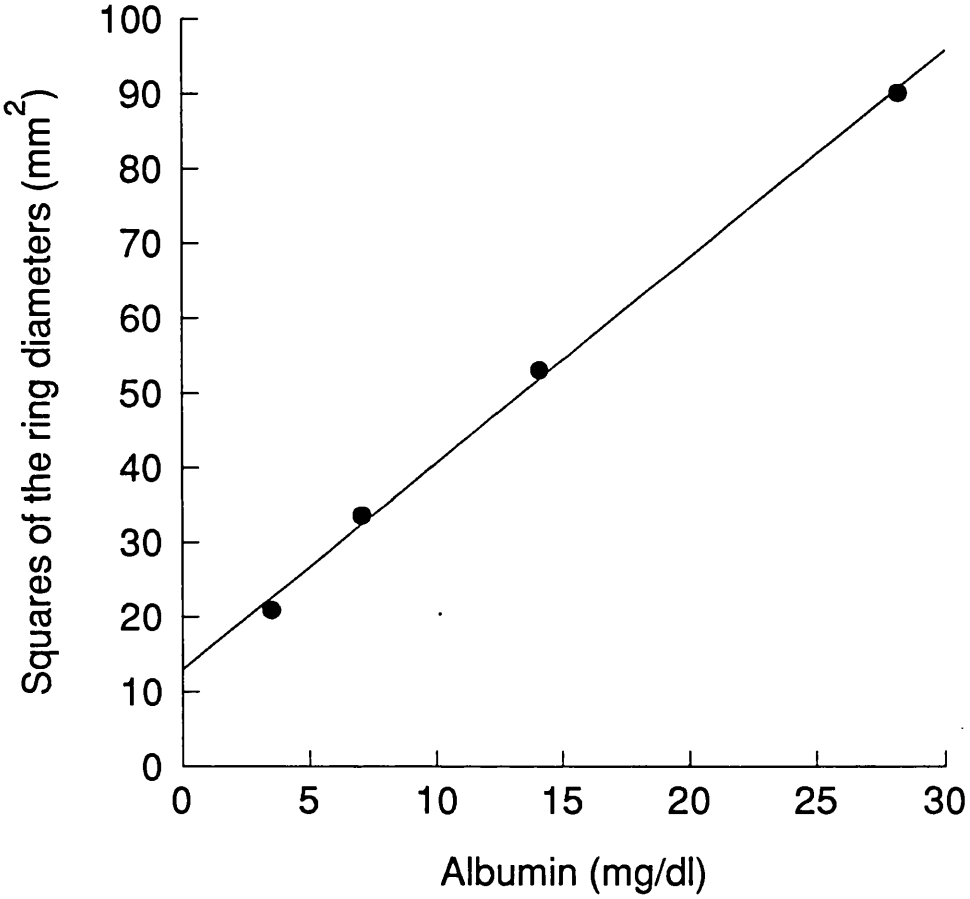


Figure 2.1 Standard curve for albumin measured by radial immunodiffusion.

2.3.2 Histamine assay

A radioimmunoassay was used for the quantitative determination of histamine in nasal lavage. The assay was based on competition between modified histamine in the sample and ^{125}I-radiolabelled histamine for binding to antibody coated on the tubes (McBride *et al.*, 1988). The assay was performed in duplicate and involved an initial acylation step on all standards, samples and tracer, in which histamine was converted into a succinyl glycinamide derivative (Morel & Delaage, 1988). This was followed by the immunoanalysis, where ^{125}I- radiolabelled histamine was added to the acylated histamine. The materials were mixed and transferred to monoclonal antibody coated tubes and incubated at 40^{0}C for 18 hours. Solutions were aspirated and discarded, then antibody coated tubes were counted for 1 minute in a gamma counter (Beckman LS 1801). The concentration of histamine in the samples was determined by interpolation on a standard curve constructed as follows:

1. The mean count for each set of duplicates was determined.

2. The mean count (B) for standards and samples was expressed as a fraction of the mean total counts (T).

3. The B/T values for the standards were plotted against the log of the histamine concentration of the standards (nM), and the quantity of histamine in the samples was determined by interpolation from the standard curve. (Figure 2.2).

The acylation step in this procedure increases the sensitivity of the assay to 0.2nM in the sample. Acylated histamine is the immunogen used to make the monoclonal antibody, and this apparently results in the production of immunogen approaching the theoretical limit of affinity and specificity for binding (Peyret *et al.*, 1986). The cross-reactivity of the assay is given below.

Analogue	% Cross-reactivity
Acylated histamine	100
Acylated t-methylhistamine	0.065
Histamine	0.0001

Table 2.1 Cross-reactivity of the histamine assay. (Immunotech Int., Marseille, France).

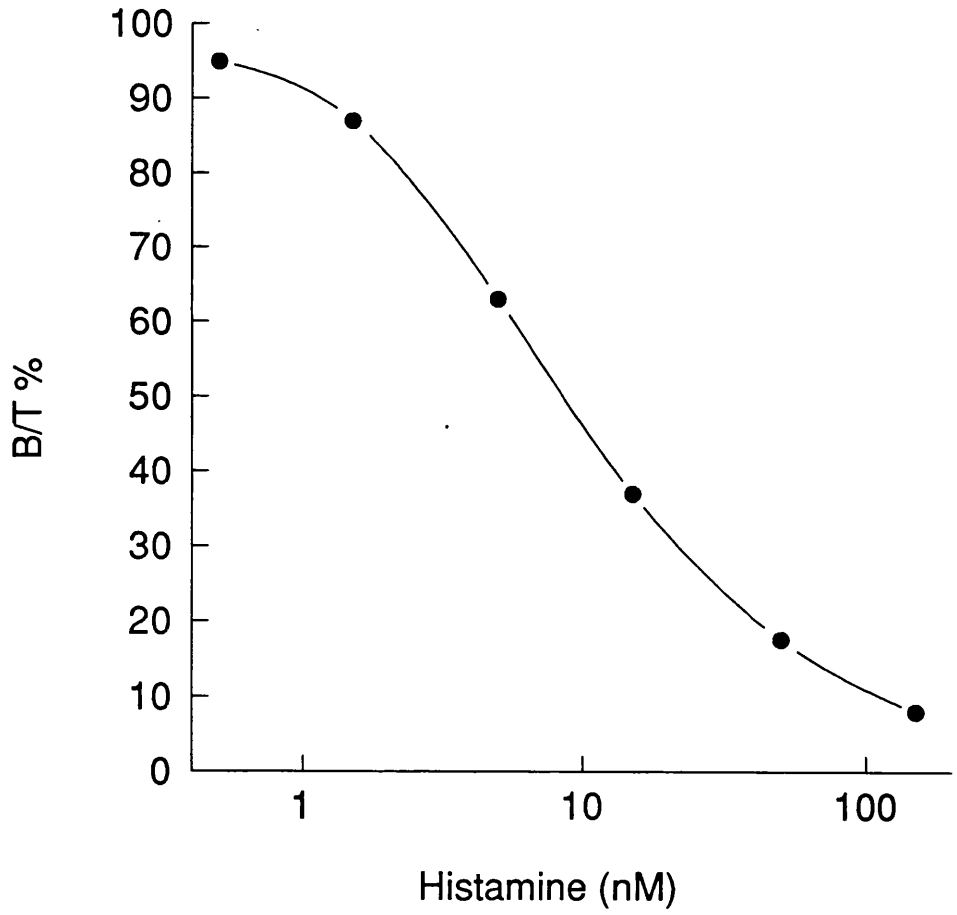


Figure 2.2 Standard for histamine assayed by radioimmunoassay.

2.3.3 ECP assay

ECP in nasal lavage was quantitatively determined by a double antibody radioimmunoassay, based on the method described by Venge *et al.*, (1977). ECP in the sample competes with a fixed amount of ^{125}I-radiolabelled ECP for the binding sites of specific antibodies. The assay was performed on duplicate samples and standards. The bound ECP-antibody complex and the free ECP were separated by addition of a second immunosorbent antibody. The samples were centrifuged and the supernatant containing the unbound ECP was decanted. The radioactivity in the pellet containing the bound ECP-antibody complex was then measured in a gamma counter (Beckman LS 1801) and is inversely proportional to the quantity of ECP in the sample. After counting, the concentration of ECP in the samples was determined from a standard curve as follows:

1. All counts were corrected to counts per minute (CPM).

2. The mean count for each set of duplicates was determined.

3. The mean count (B) for standards and samples was expressed as a percentage of the mean counts of the "0-standard" (B_0).

$$\% \text{ activity bound} = \frac{B(\text{of standard or sample}) \times 100}{B_0}$$

4. The percentage activity bound (% B/B_0) for the standards were plotted against the log of the standard ECP (μg/l), and the quantity of ECP in the samples was determined by interpolation from the standard curve (Figure 2.3).

The detection limit of this assay is $< 2\mu$g/l. Cross reaction tests showed cross-reactivity only with EPX and this was $< 0.06\%$ (Pharmacia Diagnostics, Uppsala, Sweden).

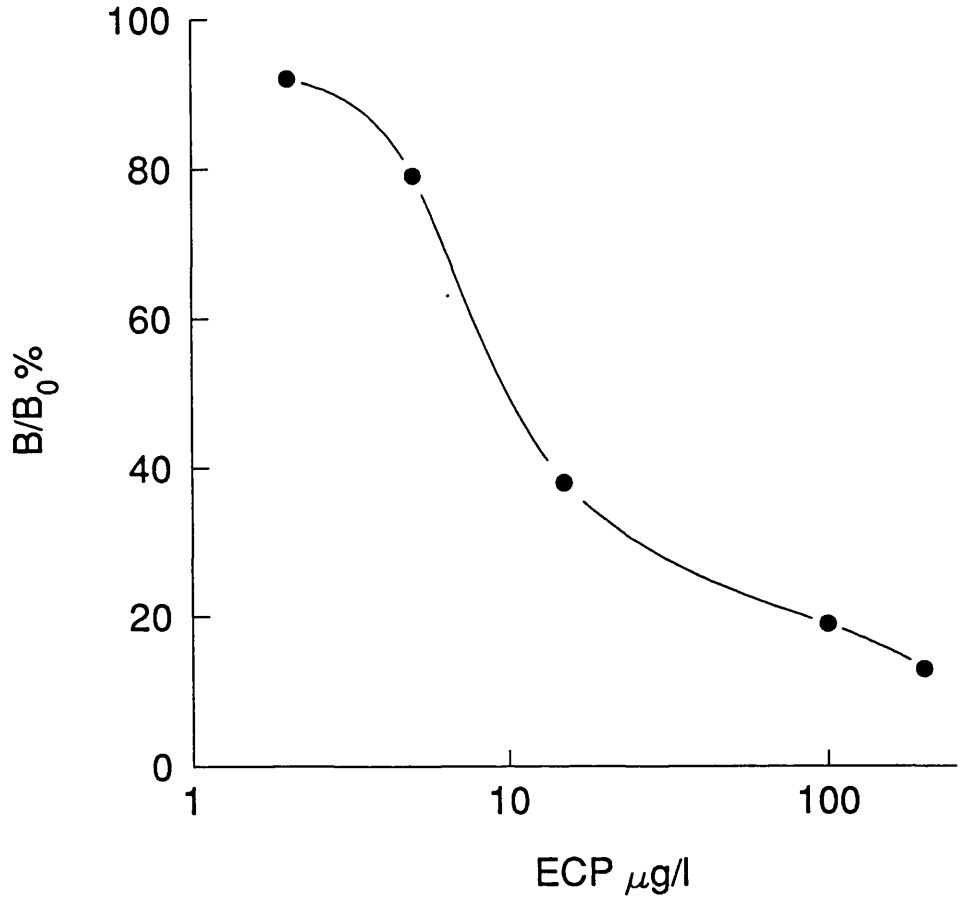


Figure 2.3 Standard curve for eosinophil cationic protein measured by radioimmunoassay.

2.3.4 Vitamin E measurements

Blood (5 ml) was obtained by superficial venepuncture of the antecubital fossa, and left to clot at room temperature. The blood was then centrifuged at 1400xg for 10 min, the serum aspirated off, then stored at -20°C until analysis.

The vitamin E was assayed the Chemical Pathology Department, University College London. This was achieved by measuring its fluorescence in hexane in the 290nm excitation, 330nm emission range. The serum samples were saponified with potassium hydroxide in the presence of ascorbic acid, as an anti-oxidant, and ethanol to reduce the extraction of interfering substances (Taylor *et al.*, 1976).

2.4 Histamine release from rat peritoneal mast cells

2.4.1 Collection of unpurified mast cells

Male Sprague Dawley rats weighing 200-300g were killed by a rising concentration of CO_2 and cervical dislocation. The peritoneal wall was exposed and 10ml of saline (0.9%w/v) containing heparin (25u/ml) was injected into the peritoneum. The peritoneal area was then massaged gently for approximately 30 seconds. The peritoneal cavity was opened and the washings were aspirated by a syringe. A further 5-10ml of heparinised saline was introduced into the peritoneum agitated and aspirated as before. The washings were pooled, then aliquoted into the required number of polystyrene conical centrifuge tubes, 1ml per tube. The washings from 1 rat were sufficient for approximately 12 reaction tubes.

2.4.2 Histamine release

The aliquoted cell suspensions were centrifuged at 200xg for 5 minutes. The supernatants were discarded and the cells resuspended in 0.9ml of HEPES-buffered Tyrode solution. The tubes were then placed in a water bath (37°C) in a timed sequence of 15 seconds and

then left to incubate for 10 minutes. Peptides were added in duplicate to the tubes following the same timed sequence, and then left for a further 10 minutes. The reaction was quenched by the addition of 4ml ice-cold incubation medium to give a 5ml final volume, and the tubes were centrifuged at 800xg for 5 minutes. The supernatants containing released histamine were decanted into another set of numbered tubes ready to be prepared for assay. Five ml incubation medium was added to each pellet containing residual histamine. The residual histamine was extracted by heating the tubes in a boiling water bath for 5 minutes and the tubes allowed to cool to room temperature.

2.4.3. Fluorimetric histamine assay

The histamine assay is adapted from that described by Shore *et al.*, (1959). Two ml or an appropriate dilution made up to 2ml, of both supernatant and residual histamine solutions, were put into another set of tubes. Three blanks containing either incubation medium alone, incubation medium + an appropriate concentration of bradykinin or incubation medium + an appropriate concentration of the other peptide involved in the particular experiment, were prepared. The fluorescence reading obtained from the blanks was subtracted from the appropriate fluorescence reading of the experimental samples. To each assay tube, 400μl of NaOH, 1M was added, and then at discrete time intervals, 100μl of o-pthalaldehyde, 10mg/ml in methanol, was added. Four minutes later, 200μl of HCl, 3M was added. All tubes were vortexed after each addition. The fluorescent product was then estimated in polystyrene cuvettes using a fluorescence spectrometer (Perkin Elmer model 3000), set at an excitation wavelength of 365nm and an emission wavelength of 450nm. Slit widths were set to give excitation and emission bandwidths of 10nm and the instrument gain was set at minimum.

2.4.4. Solutions

The mast cell incubation medium was a HEPES-buffered Tyrode's solution of the following composition (mM): HEPES (N-2-Hydroxyethylpiperazine-N'-3-ethanesulphonic acid), 20; NaCl, 136.9; KCl, 2.68; glucose, 5.55; NaH_2PO_4, 0.282; $CaCl_2$, 1. The pH of the solution was adjusted to 7.4.

2.5 *In vivo* determination of nasal vascular permeability in the guinea-pig

2.5.1 Animal preparations

Male Dunkin-Hartley guinea-pigs weighing 450-580g were used in all experiments. The animals were anaesthetised with urethane, 7mg/kg injected intraperitoneally. Additional doses were given as required to maintain adequate anaesthesia, as determined by loss of both the corneal and paw reflexes. The animals were placed on a heated blanket (Bioscience, Sheerness, Kent, U.K.) which maintained body temperature at approximately 37°C. The left carotid artery was cannulated using a polyethylene catheter, which was connected to a pressure transducer in order to monitor blood pressure. Another polyethylene catheter was inserted in to the right jugular vein for the intravenous administration of Evans blue. A tracheal cannula was inserted into the lumen of the caudal trachea, secured with a suture and connected to a mechanical ventilator (Harvard model 50-1718, Harvard Apparatus Ltd., Edenbridge, Kent, U.K.) set at 51 strokes/minute.

A perfusion circuit was developed to include the nasal passages and a section of the trachea (Figure 2.4). A polyethylene cannula (5cm long) was inserted to a depth of 2-3cm into the cephalic end of the trachea. Polyethylene cannulas (5cm long) were inserted to a depth of 0.5-1cm into both nasal passages and held in position with glue. The nasal cannulas and tracheal cannula were connected with polyethylene tubing via a perfusion pump (Watson-Marlow model 501, Watson-Marlow, Falmouth, U.K.) to form a closed circuit. The buccal cavity was sealed with glue thus ensuring that air could not enter and perfusion fluid could not escape.

The circuit was filled with saline, 2.5 ml which was perfused at 10ml/min. At intervals the perfusion circuit was emptied, the saline (containing the plasma exudate) collected and the circuit refilled. The first 5 collections taken over 25 minutes after the perfusion circuit was established were discarded. Evan's blue dye, 20mg/kg was injected intravenously and a 5 minute sample collected, followed by saline and kinin challenge.

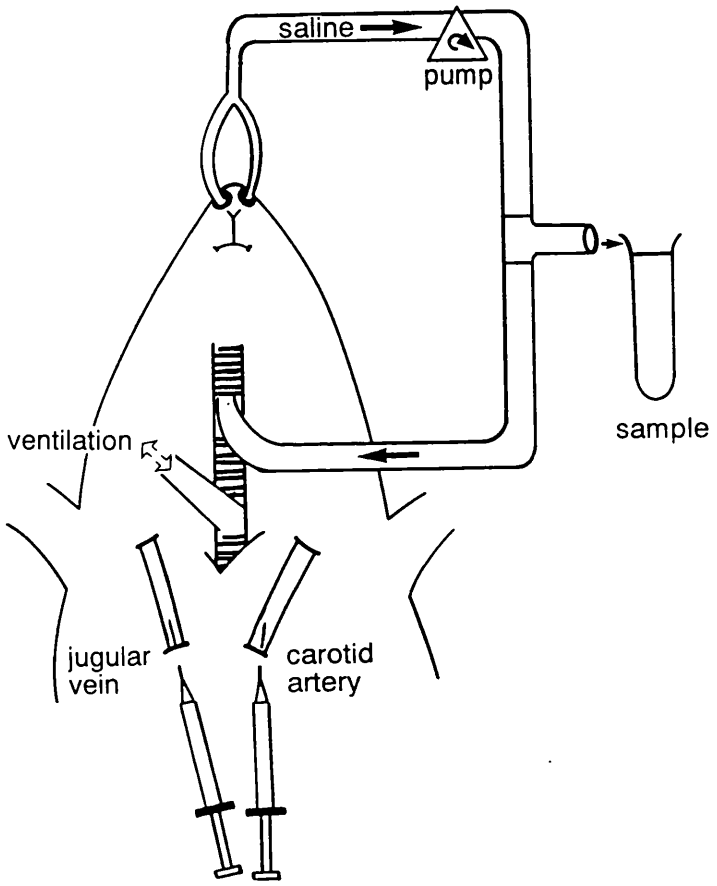


Figure 2.4

In vivo method to measure changes in vascular permeability in the anaesthetised guinea-pig.

Evan's blue dye is injected intravenously and its concentration following kinin challenge is determined in perfusion fluid collected from a closed circuit encompassing the upper airway including the nasal passages and a section of tracheal lumen.

2.5.2 Measurement of vascular permeability

Samples of perfusate (1ml) were centrifuged at 1500g for 10 minutes and Evans blue concentration in the supernatant was determined spectrophotometrically at 610nm (Anthos HT3 spectrophotometer, Anthos Labtec Instruments, Salzburg, Austria). Evans blue leakage was quantified by interpolation on a standard curve of dye concentration in the range 0-10μg/ml (Figure 2.5) and expressed as μg/ml of perfusate.

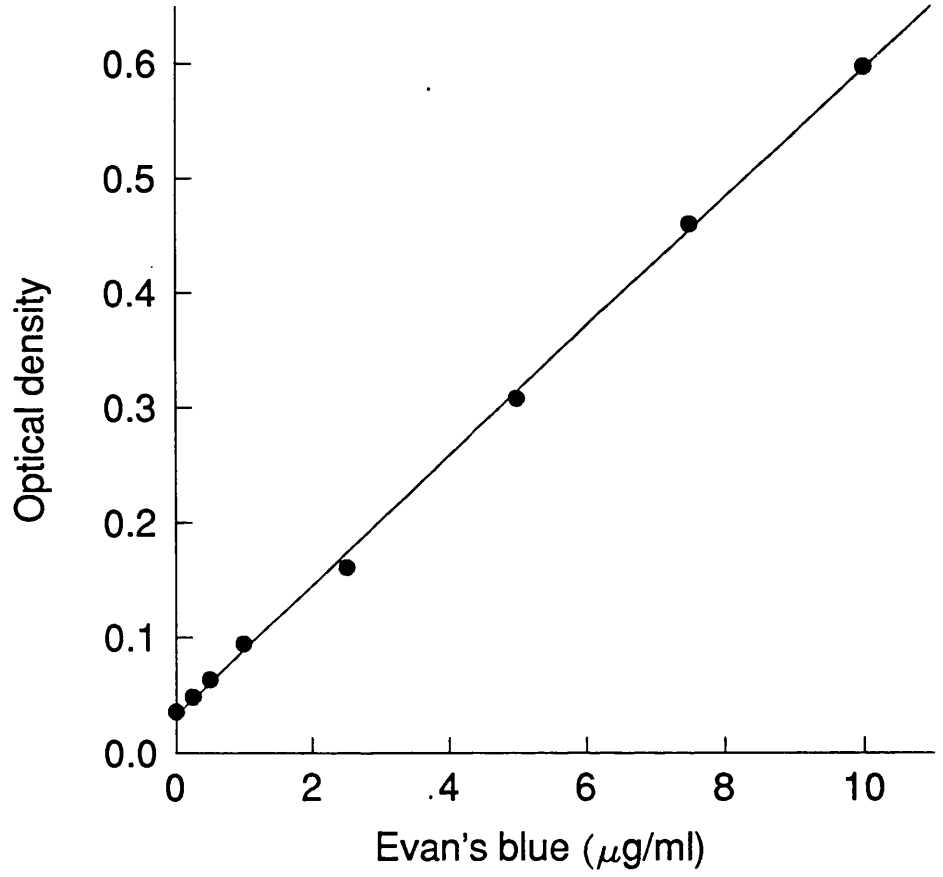


Figure 2.5

Standard curve for Evan's blue concentration.

CHAPTER 3

A comparison between acoustic rhinometry and posterior rhinomanometry in the measurement of nasal airway patency.

3.1 Introduction

Changes in the patency of the nasal airway leading to nasal blockage is one characteristic of rhinitis. The ability to quantify this is of importance for both in the experimental situation, such as in the evaluation of pharmacological or allergen challenges; and also in the objective clinical assessment of upper airway diseases.

Several methods for assessing nasal airway patency have been described. Early attempts to examine nasal respiration were rather qualitative, and were based on the principle that water vapour is condensed from exhaled air on a cold mirror held in front of the nose (Zwaardemaker, 1891). More quantitative assessments have since been made using methods such as rhinomanometry (Kern, 1973), nasal inspiratory peak flow (Holmstrom *et al.*, 1990), apparent nasal volume (Gleeson *et al.*, 1986) and body plethysmography (Nolte & Luder-Luhr, 1973). However, all these methods have some disadvantages. A high degree of subject co-operation is required and the methods can be time-consuming and often unsuitable for subjects with severely congested nasal airways. To date, no single method has gained general acceptance, which reflects the difficulty of obtaining reproducible and valid measurements of nasal airway patency. Thus, there is a requirement for a simple, non-invasive and reliable technique requiring minimal subject compliance. Such a technique should be standardized among investigators, to provide confidence in the measurement.

More recently, the method of acoustic rhinometry has been introduced. This evaluates the geometry of the nasal cavity, thus providing information on the patency. It has a number of potential advantages over other methods, and it seemed useful, therefore, to compare acoustic rhinometry and a more established method for quantifying nasal

patency, such as posterior rhinomanometry.

Acoustic rhinometry

Acoustic rhinometry is based on acoustic reflection (Jackson *et al.*, 1977). It provides a two-dimensional picture of nasal cross-sectional area as a function of distance from the nostril. An acoustic impulse directed into the airway is reflected by local changes in acoustic impedance resulting from changes in cross-sectional area of the nasal cavity. It is possible to determine the distance to the local impedance change and also the size of the change in cross-sectional area, by comparing incident and reflected waves (Jackson *et al.*, 1977). Measurements of cross-sectional area at specified distances are provided, and by integrating the area under the area-distance curve over a specified distance, nasal cavity volumes can be obtained.

Posterior rhinomanometry

Posterior rhinomanometry provides a measure of nasal resistance to airflow. It involves sampling the pressure in the oropharynx, using an oral pressure cannula, and assuming that this pressure is the same as posterior nasal pressure. In this way, pressure for both nasal passages is measured simultaneously. Flow through both nostrils together is also measured in order to calculate resistance. Resistance is the reciprocal of patency and is defined as the quotient of pressure (p) and flow (V), i.e. $R=p/V$. Resistance is expressed in units of $Pa.cm^{-3}.sec$. Resistance is calculated at a fixed pressure. We have used a reference pressure of 75Pa for posterior rhinomanometry. The reference pressure should fall on the linear part of the pressure-flow curve since the relationship between pressure and flow is somewhat complex due to turbulent airflow and the pressure-flow plot is curvilinear.

In general when conducting measurements of nasal function, the least invasive method is likely to produce the most reliable result because the nasal mucosa is relatively reactive. In this context, the physiological factors controlling and influencing nasal

patency are briefly considered.

Mechanism for change in nasal patency

Changes in nasal airway patency are associated with a change in vascular tone, in contrast to the lower airways where bronchial smooth muscle contraction can also be involved. The nasal mucosa is capable of enormous physiological and pathological variations in thickness due to its vascularity and to the looseness of the submucosa. The vascular supply to the nose is supplied by the internal and external carotid arteries. Erectile tissues in the form of cavernous sinusoids are located between the capillaries and venules. The sinusoids are surrounded by circular and longitudinal smooth muscle, innervated by the autonomic nervous system. They are usually constricted by a continuous sympathetic stimulation, but they can dilate when tone is lost, or when parasympathetic stimulation produces vasodilatation. The concentration of sinusoids is paticularly marked in the mucosa covering the nasal septum and the turbinates. The anatomy of the nose is such that the nasal passages are surrounded by rigid bony walls, so that any increase in blood volume reduces the cross-sectional area and volume of the air passages and leads to an increase in nasal airway resistance.

Factors that influence nasal patency

Factors which modify nasal patency, are mostly considered to be reflex in nature, rather than being localised. The nose contains both the afferent and efferent components of several reflexes. Both hot and cold thermal stimuli affect the degree of congestion, as demonstrated by breathing hot and cold air (Salman *et al.*, 1971). Humidity (Ingelstedt & Ivstam, 1951) and posture (Rundcrantz, 1964) also affect nasal patency, and exercise has been shown to increase nasal patency (Richerson & Seebohm, 1968), consistent with the need for increased airflow during exercise. This effect is mediated by sympathetic nerve discharge (Richerson & Seebohm, 1968).

Therefore, with these points in mind, the purpose of this study was to assess the potential of acoustic rhinometry as a means of quantifying changes in nasal patency induced by histamine and bradykinin, and to compare this technique with the established and frequently used method of posterior rhinomanometry.

3.2 Experimental protocol

On each occasion subjects were first challenged with saline, then NAR measurements or acoustic reflection measurements were performed, every 5 minutes over a 15 minute period. The averaged data from all time points formed the baseline.

In the first study, ten subjects were given histamine, 100, 300 and 1000μg to each nostril, and NAR measurements were performed at 2, 5, 10, 15 and 20 minutes. Each subject had three doses in total, and NAR was allowed to return to baseline before proceeding with the next dose. At least one week later, subjects were given the selective H_1-antagonist, cetirizine 10mg, orally, three hours before repeat histamine challenges of 100, 300 and 1000μg. NAR measurements were then made as before. A further ten subjects were given histamine, 100, 300 and 1000μg to each nostril, and acoustic reflection measurements were performed at 2, 5, 10, 15 and 20 minutes. One week later, these subjects repeated the experiment in the presence of cetirizine, 10mg, given orally 3 hours before.

In another study, ten subjects were given bradykinin, 100, 300 and 1000μg per nostril and NAR measurements were made at 2, 5, 10, 15 and 20 min. Each subject had three doses in total, allowing NAR to return to baseline between each dose. This experiment was repeated in these subjects one week later using acoustic rhinometry to quantify nasal blockage. Again, measurements were made at 5 minute intervals during the 20 minute post-challenge period.

Data analysis

To achieve comparability, the mean of the acoustic reflection measurements from the left and right nostrils were calculated for each measurement taken, because NAR measurements made by posterior rhinomanometry measure resistance for both nostrils together. Changes in NAR and in the values obtained from acoustic rhinometry were expressed as the percentage change from saline control values to normalize the data. Responses to histamine and bradykinin were quantified for each dose as the *maximal* percentage change during the post-challenge measurements. All data was expressed as mean $\pm$ standard error of mean. Repeated measures analysis of variance (ANOVA) was used to statistically evaluate the responses to histamine and bradykinin. A probability value of $p < 0.05$ was considered significant.

3.3 Results

3.3.1 Reproducibility and sensitivity of the measurements

The coefficient of variation (CV) for mean values of each parameter measured was calculated in order to provide information about relative reproducibility. Table 3.1 demonstrates the reproducibility in one subject, who had 8 measurements taken (all parameters) over 15 minutes under baseline conditions. It shows that the coefficients of variation for each parameter are generally quite small; approximately between 4% and 8%, taking into account both nasal passages. This is with the exception of the CV's for the volume measurements corresponding to the area between 16.2cm and 20cm along the nasal airway, and the cross-sectional area measurements at 11cm. These latter values displayed a larger variability. The parameter displaying least variation between measurements was the minimum nasal cross-sectional area (A min.), for which the mean CV for right and left nasal passages was approximately 4%, and less than the CV for any of the other parameters measured by the acoustic rhinometer. For NAR, the corresponding CV of the mean values under baseline conditions, using subjects in the histamine study, was 9.6% ($n=10$).

The sensitivity of the parameters given by the acoustic rhinometer compared with measurements of NAR was also considered. Figure 3.1 shows that volume measurements of the nasal cavity appear to be less sensitive than measurements of NAR to histamine-induced changes in nasal patency. However, measurements of NAR and A min., recorded after an identical histamine challenge in another subject, appeared to be of a similar magnitude (figure 3.2).

3.3.2 Comparison of acoustic rhinometry and posterior rhinomanometry in quantifying dose-response relationships

Histamine, 100-1000μg in each nostril, produced a dose-related increase in NAR and a dose-related decrease in A min. Figure 3.3 is an example of an area-distance function provided by the acoustic rhinometer pre- and post-histamine challenge, 1000μg, demonstrating that nasal blockage occurs throughout the nasal mucosa following such a challenge. Figure 3.4 is an example of a pressure-flow plot from the rhinomanometer obtained after an identical challenge. Figure 3.5 shows that histamine, 100, 300, 1000μg per nostril, resulted in increases in NAR of 69.7%, 126.5% and 174.3% respectively, (ANOVA $p=0.002$, $n=10$), and figure 3.6 demonstrates that over the same dose range, histamine produces dose-related decreases in A min. of 27.8%, 36.1%, and 53.8% respectively, (ANOVA $p=0.028$, $n=10$).

After pretreatment with cetirizine, 10mg, histamine 100, 300, 1000μg per nostril, resulted in increases in NAR of 21.0%, 15.2% and 23.3% respectively, $n=10$ (figure 3.5), and decreases in A min. of 3.0%, 19.6% and 11.8%, $n=10$, (figure 3.6). None of these responses to histamine in the presence of cetirizine, 10mg were statistically significantly different from measurements taken at baseline (saline challenge).

Subjects challenged with bradykinin 100, 300 and 1000μg per nostril, all experienced an increase in nasal blockage. Figure 3.7 shows bradykinin-induced increases in NAR, of 48.1%, 86.2% and 186.4% respectively, (ANOVA $p=0.020$, $n=10$), and figure 3.8 represents bradykinin-induced decreases in Amin. These were 16.4%, 28.7% and 49.3%

respectively, (ANOVA,$p < 0.0001$, $n = 10$).

Pooling data from experiments where histamine or histamine in the presence of cetirizine were used, and only using paired data, i.e where the same subject had received identical challenges, after which, NAR had been measured once and A min. on the other occasion) the correlation between the two methods was examined. Figure 3.9 shows linear regression analysis of this data, which identifies a significant negative correlation ($p < 0.0001$) between the magnitude of change in A min compared with the magnitude of the change in NAR after challenge.

Parameter- Volume (cm^3) Area (cm^2)	Right			Left		
	Mean	SD	CV	Mean	SD	CV
Vol (6.9, 14.1)	13.76	0.694	0.050	6.08	0.390	0.064
Vol (9.0, 14.8)	15.19	0.849	0.056	5.89	0.491	0.083
Vol (16.2, 20)	39.09	5.203	0.133	13.83	2.125	0.154
Area (8.3)	0.99	0.072	0.072	1.00	0.038	0.038
Area (10.3)	2.06	0.123	0.060	0.72	0.044	0.060
Area (11.0)	2.25	0.472	0.210	0.68	0.086	0.125
Area (13.4)	2.77	0.200	0.072	1.38	0.116	0.084
A min.	0.49	0.012	0.026	0.26	0.017	0.065

Table 3.1

Reproducibility of each parameter obtained from acoustic rhinometry.

Means, standard deviations (SD) and coefficients of variation (CV) for calculated volumes and areas at specified distances along the nasal passages. The data is from 8 measurements taken from one subject using the acoustic rhinometer. Acoustic reflection measurements were made over a 15 minute period under baseline conditions.
Parameters: Vol (6.9,14.1) refers to the volume between 6.9cm and 14.1cm along the airway from the nostril etc.; Area (8.3) refers to the cross-sectional area of the airway measured at 8.3cm from the nostril etc.; A min. = minimum nasal cross-sectional area. Means and standard deviations are expressed as cubic centimetres and square centimetres, as appropriate.

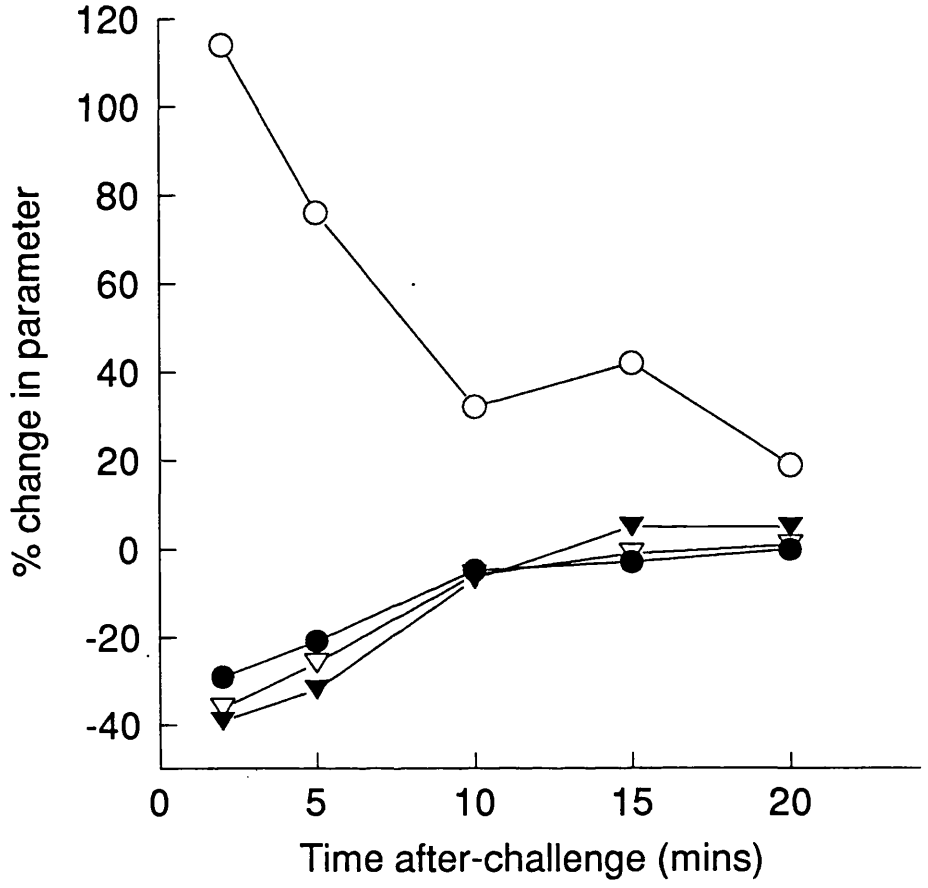


Figure 3.1

Relative sensitivity of nasal volume changes and changes in NAR.

Responses to histamine, 100μg in one subject expressed as % increases in NAR above control (saline) and % decreases in nasal volume from the control.
(○)- NAR; (●)- volume (6.9, 14.1) i.e. volume between 6.9cm and 14.1cm along nasal passage; (▽)- volume (9, 14.8); (▼)- volume (16.2, 20).
Baseline (saline) value for NAR was 0.267 Pa.s.cm^{-3}; for volume (6.9, 14.1) it was 10.25cm^2; for volume (9, 14.8) it was 11.09cm^2; and for volume (16.2, 20) it was 25.4cm^2.

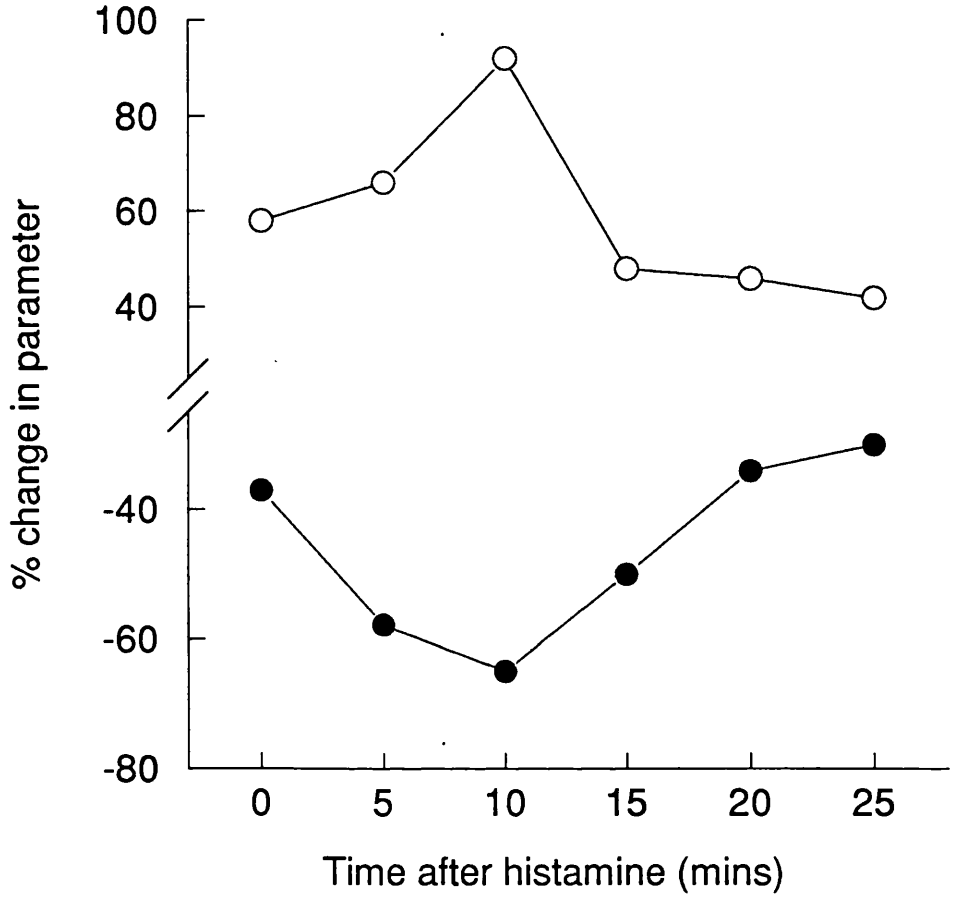


Figure 3.2

Relative sensitivity of changes in A min. and changes in NAR.

Responses to histamine, 100μg in one subject expressed as percentage changes from control values. (○)- NAR; (●)- A min. Baseline (saline) value for NAR was 0.224 Pa.s.cm^{-3}; and for A min. it was 0.69cm^2.

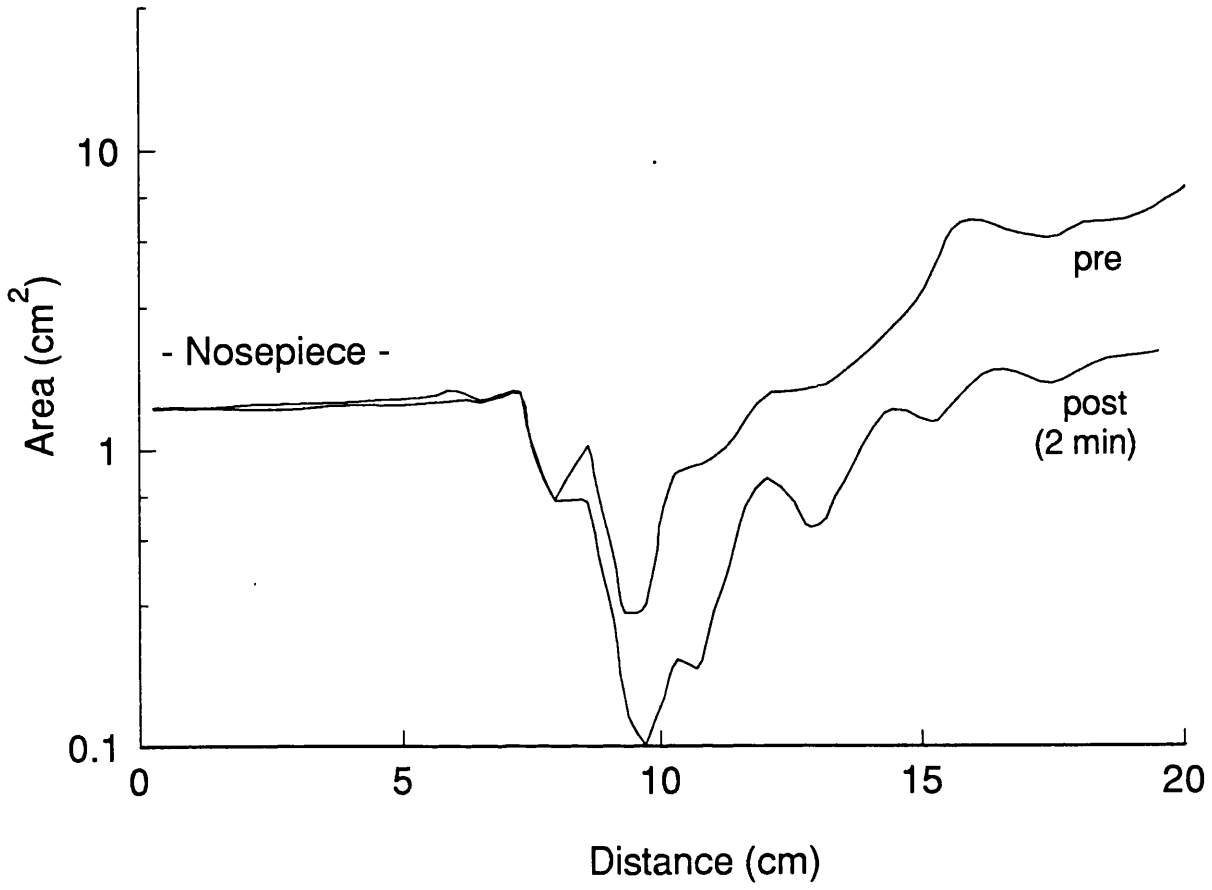


Figure 3.3

Cross-sectional area of the nasal cavity as a function of distance from the nostril obtained by acoustic rhinometry.

The area-distance curves illustrate a normal phase during the physiological nasal cycle, as well as the result of a pharmacological challenge with histamine, 1000μg. The initial value for the minimum nasal cross-sectional area (A min.) was 0.32cm^2, changing to 0.1cm^2, 10 min after challenge.

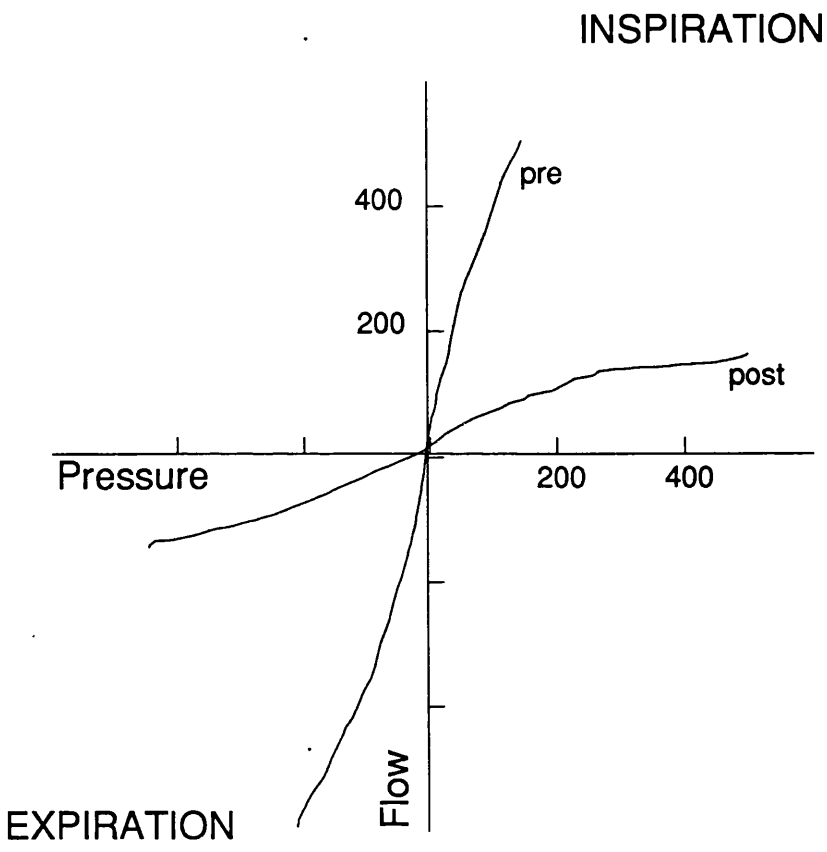


Figure 3.4

Nasal airway resistance measured by posterior rhinomanometry.

Flow-pressure curves obtained pre- and post-challenge with histamine, 1000μg. In order to calculate nasal airway resistance (NAR), the nasal airflow is measured at a sample pressure of 75Pa. The mean NAR for inspiration and expiration before challenge was 0.272 Pa.s.cm^{-3}, changing to 0.912 Pa.s.cm^{-3} measured 10 min post-challenge.

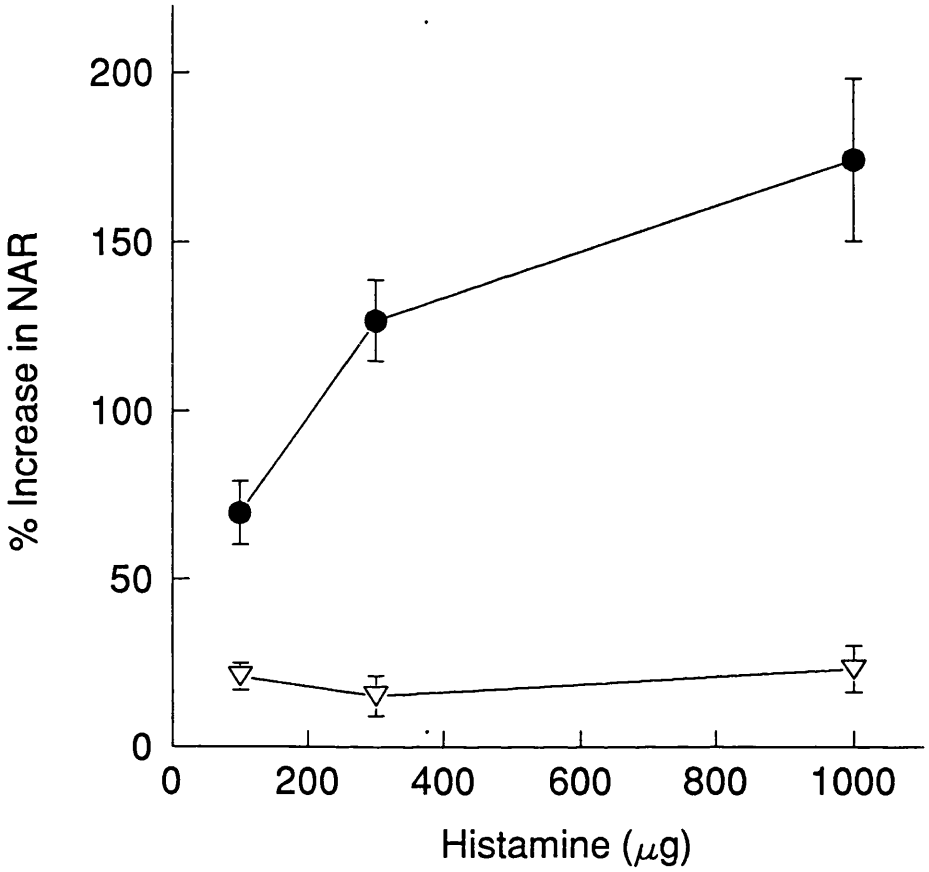


Figure 3.5

Dose-response curve for histamine using active posterior rhinomanometry before and after cetirizine treatment.

Responses are expressed as maximal percentage increases in nasal airway resistance (NAR) during the post-challenge period from control (saline) values. (●) - histamine; (▽) - histamine in the presence of cetirizine, 10mg. The data are the means ± s.e. m from 10 seperate experiments for both groups. Control values for NAR were 0.366 ± 0.027 Pa.s.cm^{-3} for histamine and 0.392 ± 0.033 Pa.s.cm^{-3} for histamine/cetirizine.

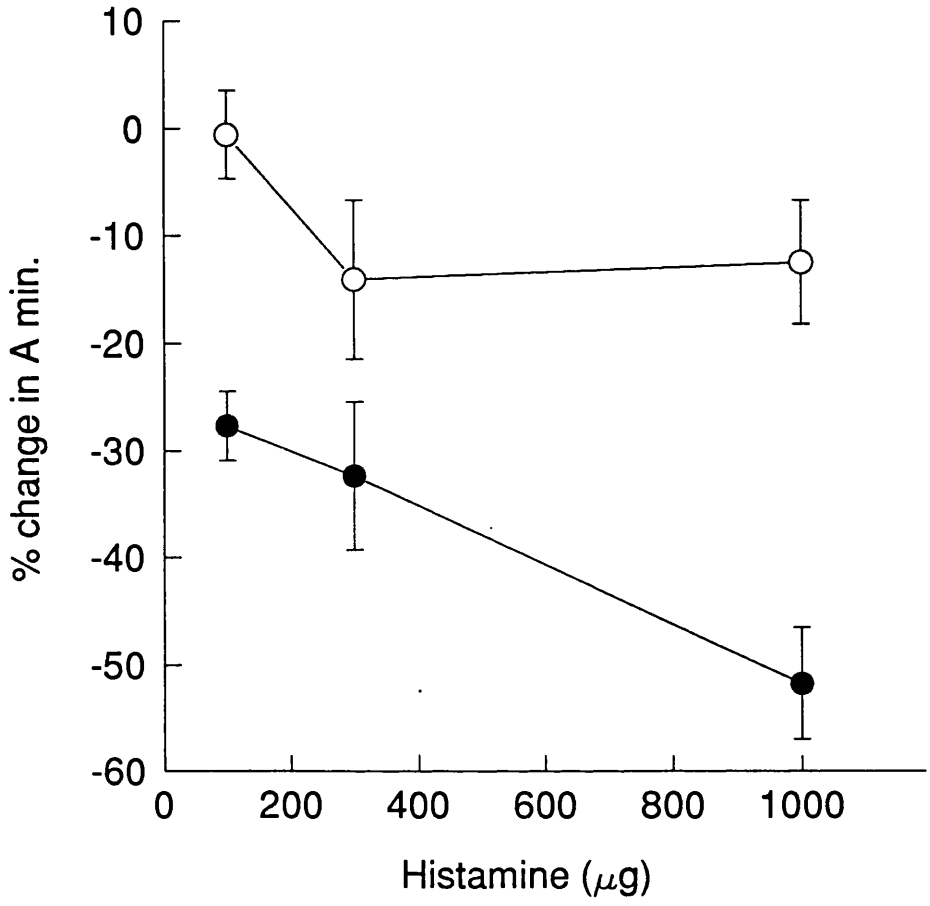


Figure 3.6

Dose-response curve for histamine using acoustic rhinometry before and after cetirizine treatment.

Responses are expressed as the percentage decrease in the minimum nasal cross-sectional area (A min.) from the control values. (●) -histamine; (○) - histamine in the presence of cetirizine, 10mg. The data are the means ± s.e.m from 10 seperate experiments for both groups. Control (saline) values for A min. were 0.462 ± 0.019 cm^2 for histamine and 0.446 ± 0.028 cm^2 for histamine/cetirizine.

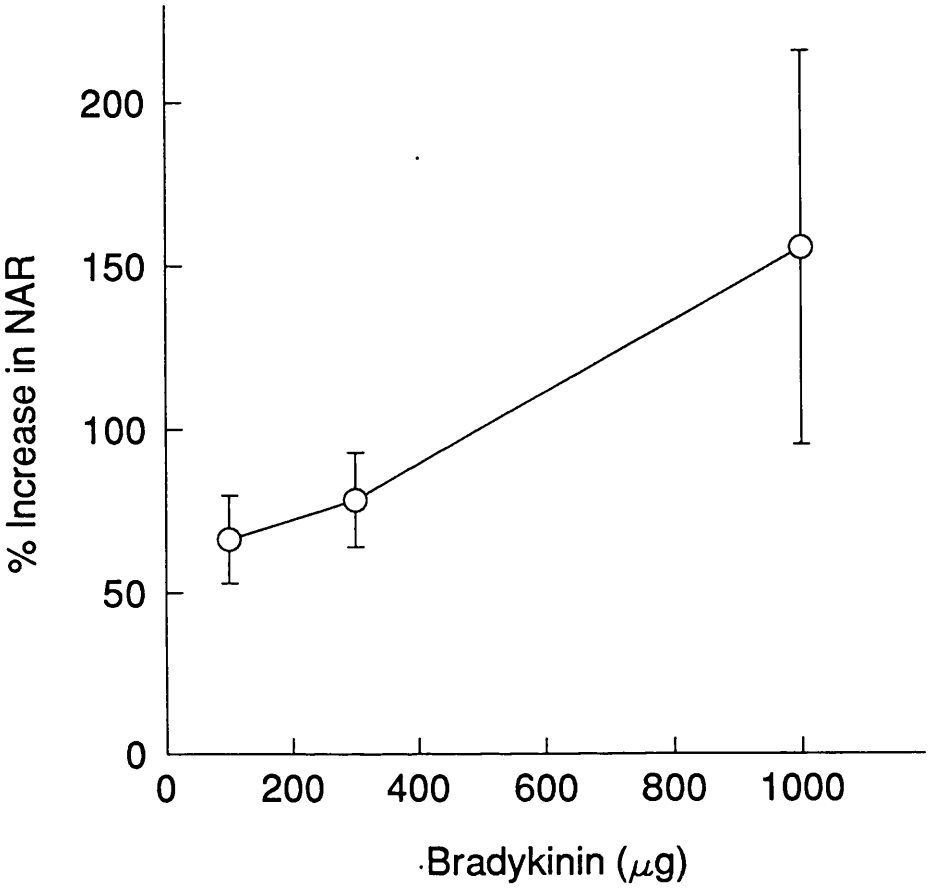


Figure 3.7

Dose-response curve for bradykinin using active posterior rhinomanometry.

Responses are expressed as maximal percentage increases in nasal airway resistance (NAR) during the post-challenge period from control (saline) values. The data are the means from 10 seperate experiments. Vertical error bars represent s.e.m except where they fall within the limits of the actual data point. Control values for NAR were 0.311 $\pm$ 0.016 Pa.s.cm^{-3}.

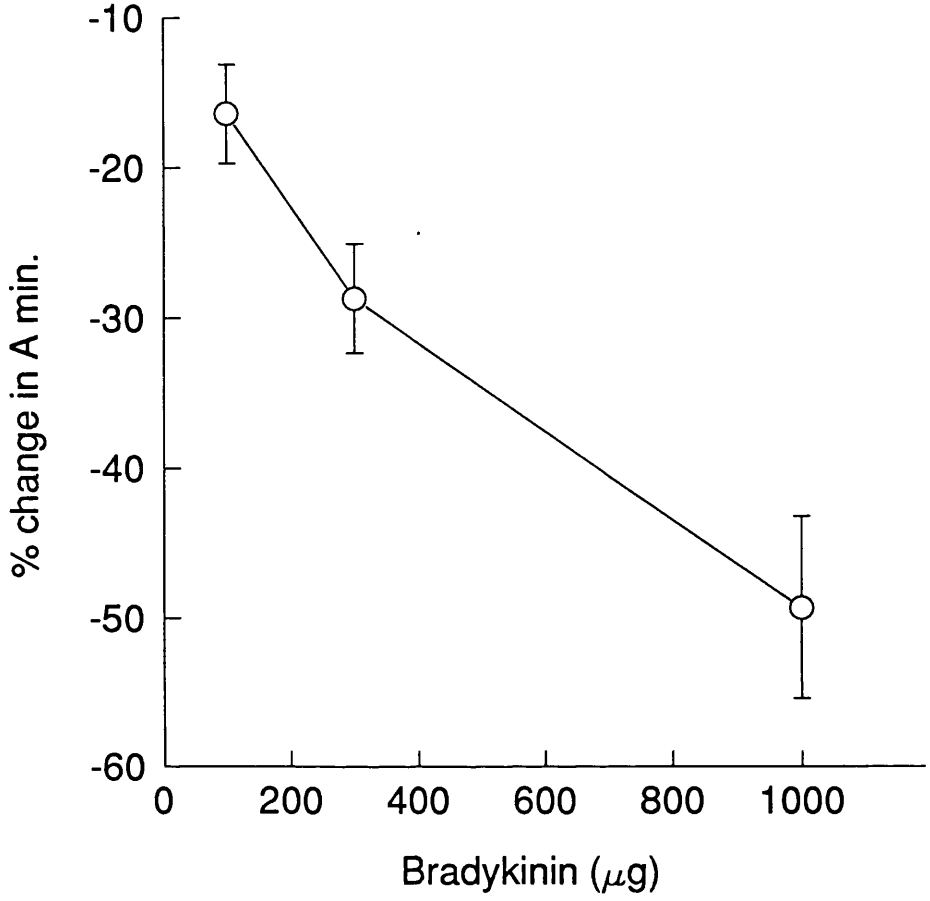


Figure 3.8

Dose-response curve for bradykinin using acoustic rhinometry.

Responses are expressed as the percentage decrease in the minimum nasal cross-sectional area (A min.) from the control values. The data are the means ± s.e.m from 10 seperate experiments for both groups. Control (saline) values for A min. were 0.467 ± 0.034 cm^2.

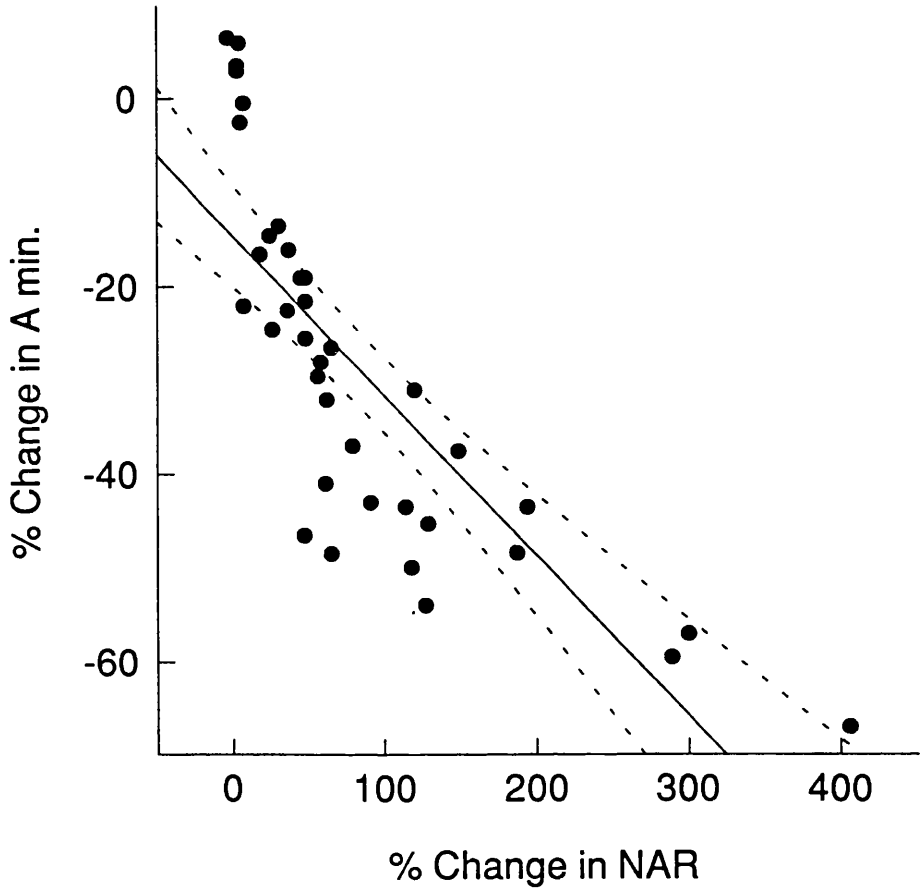


Figure 3.9

Correlation between measurements of nasal blockage obtained by posterior rhinomanometry (NAR) and acoustic rhinometry (A min.).

In total 36 parallel measurements are shown. The data are from studies where histamine challenge was performed alone, or in the presence of cetirizine. All data points represent measurements of NAR and A min. after identical challenges in the same subject. According to linear regression analysis, there is a significant negative correlation between the two methods, ($r=-0.80$, $p<0.0001$). 95% confidence limits are also shown.

3.4 Discussion

The data from this study show that it is possible to obtain reproducible dose-response relationships for histamine and bradykinin using acoustic rhinometry. The effects of histamine and bradykinin on acoustically determined changes in nasal airway patency are demonstrated here for the first time and these effects have been compared with changes in patency determined by posterior rhinomanometry.

The method of posterior rhinomanometry was chosen as an established method with which acoustic rhinometry could be compared. Rhinomanometry has probably received more attention and has been used most frequently in the past. It has, without doubt, a number of drawbacks, but a good correlation between posterior rhinomanometry and body plethysmography has been shown (Nolte & Luder-Luhr, 1973). Body plethysmography is said to be the standard method of measuring resistance giving the most accurate results (Nolte & Ulmer, 1966). Therefore, it seemed reasonable to select posterior rhinomanometry for this comparison.

Histamine and bradykinin were chosen for the challenge agents in this study, since the effects of histamine on the nasal airway are well established (Britton *et al.*, 1978; Corrado *et al.*, 1987), as are those of bradykinin (Proud *et al.*, 1988; Rajakulasingam *et al.*, 1991). The histamine- and bradykinin-induced changes in NAR shown, agree with previously established effects of these agents, and correlate with the measurements obtained in this study by acoustic rhinometry. The histamine-induced changes in nasal patency have been blocked with cetirizine, 10mg and this is in accordance with other studies investigating the effect of H_1-receptor antagonists on nasal responses (Bousquet *et al.*, 1988; Horak *et al.*, 1993).

The acoustic rhinometer was programmed to provide four measurements of nasal volume between different specified distances in the nasal cavity, and measurements of cross-sectional area at four defined distances into the airway, together with a minimum nasal cross-sectional area (A min.). Initially, all parameters were considered. However it became apparent, once the data had been normalised (expressed as percentage changes

from control) that the magnitude of the volume changes induced by histamine or bradykinin was small. At high doses, subjects complained of blockage, but as the data shows (figure 3.1), this was not reflected in a very large volume change. Also, the magnitude of the change in NAR following histamine or bradykinin challenge was greater. It seemed, therefore, that nasal volume was relatively insensitive to drug-induced changes, and the probable explanation for this is that changes in volume over a given distance in the nasal airway tend to be of lesser magnitude than changes in resistance to flow. Therefore, an obstruction, such as might be caused by a small change in vascular tone, means that flow is affected and hence resistance changes, but such a vascular response does not cause the volume of the cavity to change appreciably. Also, the variability of some of the volume changes was relatively high. This probably reflects the larger dimension and variation of the airway in the regions being measured.

The use of cross-sectional areas for expressing the results was then considered. The minimum cross-sectional area (A min.) was selected from those provided by the acoustic rhinometer. This exhibits less variability than the cross-sectional areas at specified distances along the airway. Anatomical differences between individuals is such that nasal structures such as the turbinates, which influence the dimensions of the airway, are located at different distances along the nasal passage so, structurally, the actual airway measured at 10cm for example in one individual could be quite different from that in another. The use of A min. seemed more reasonable and least likely to exclude information or give rise to insensitivity. The studies revealed that A min. was, infact, the most sensitive measurement, and this probably reflects the fact that the limiting factor with respect to nasal resistance to airflow is the dimension of the narrowest part of the airway. The A min. values also exhibited more consistent baseline values, as revealed from estimates of the coefficient of variation of the mean values.

Acoustic reflection measurements have previously been used for *in vivo* examination of the trachea (Jackson & Krevans, 1984), the pharnyx (Brown *et al.*, 1986) and the supraglottal oral cavity (Sondhi & Gopinath, 1971). Acoustic response investigations of the human trachea have been shown to be reproducible and correlate with equivalent areas determined by X-ray examination (Brooks *et al.*, 1984) and from computerized

tomographic (CT) scans (D'Urzo *et al.*, 1987). Hilberg *et al.* (1989) validated the acoustic technique for use on the nasal cavity, with the aim of achieving a method which would provide objective measurements of the nasal passage without the technical complications associated with other methods (Kern, 1973). In doing so, a simple and quick method requiring minimal subject input has been introduced. Acoustic rhinometry is not dependent on airflow through the nose and is, therefore, suitable even for severely congested subjects. The only potential problem with this method was that the area beyond a severe blockage or inflammed area may not be accurately estimated. However, in response to this concern, Hilberg and co-workers (1989) showed that placing orifices of up to $0.5cm^2$ in the entrance of the nasal cavity in a cast model, had no effect on the measurement in the more distal portions of the area-distance function. The same group have also shown that acoustic rhinometry can be used successfully where pathological obstruction is evident. The outline, using acoustic rhinometry, of a tumour in the nose which virtually occluded one nostril, accurately reflected the findings on clinical examination.

In contrast, rhinomanometry is time consuming, requires technical expertise, a high degree of subject co-operation and is impossible in subjects with severely congested nasal airways. There are reports that between 25% and 50% of untrained subjects cannot produce satisfactory curves when examined by rhinomanometry (Kortekangas, 1972; Kern, 1973). With those subjects where the technique is possible, the positioning of the mask appears to be critical in achieving some degree of reproducibility.

Acoustic rhinometry appears to be a useful technique in assessing the pharmacology of the nasal airway and has a number of advantages over rhinomanometry. It appears to provide reproducible information on the geometry of the nasal cavity and, being entirely non-invasive, it does not initiate any reflex control mechanisms in the nose. It should be pointed out, however, that acoustic rhinometry is essentially a measurement of anatomical dimensions of the nasal cavity whereas posterior rhinomanometry is a functional measurement based on pressure and flow in the nasal cavity. It would seem that although rhinomanometry probably still has a place as a research tool, acoustic rhinometry is the more practicable of the two, and also has the distinct advantage of providing information

about the likely distribution of resistance.

SUMMARY

- Acoustic rhinometry is a relatively new method for objectively assessing nasal airway patency. In this study we compare acoustic rhinometry with active posterior rhinomanometry.

- Twenty normal healthy volunteers underwent nasal challenge with either histamine or bradykinin, 100μg to 1000μg, and responses were assessed by acoustic rhinometry. A further 20 subjects received identical nasal challenges and responses were assessed by active posterior rhinomanometry.

- On a subsequent occasion, the subjects challenged previously with histamine, were given the selective H_1-antagonist, cetirizine, 10mg orally, 3 hours before repeat nasal challenge with histamine, 100-1000μg. Again, responses were assessed by active posterior rhinomanometry and acoustic rhinometry.

- The acoustic reflection measurements and the nasal airway resistance measurements showed comparable, significant dose-related changes in nasal patency to both histamine and bradykinin. In addition, pretreatment with cetirizine blocked the histamine-induced change in nasal patency as measured by both acoustic rhinometry and posterior rhinomanometry.

- It can be concluded that acoustic rhinometry has a number of advantages over posterior rhinomanometry. It is quick to perform, requires minimal subject co-operation and gives a reliable objective measurement of dose-related changes in nasal airway patency before and after pharmacological treatment.

CHAPTER 4

Pharmacological actions of bradykinin and bradykinin analogues in the human nasal airway. Part I: Normal subjects.

4.1 Introduction

The known pharmacological properties of the kinins, bradykinin and lys-bradykinin (kallidin), have led to these potent vasoactive peptides being implicated in the pathogenesis of various inflammatory conditions, including allergic airway diseases (Kellermeyer & Graham, 1968; Marceau *et al.*, 1983). The first indication that kinins may be involved in allergic rhinitis was the demonstration that kinin-like activity was present in nasal secretions from ragweed-allergic subjects after local antigen challenge (Dolovich *et al.*, 1970). The nasal secretions were bioassayed and kinin-like activity was identified using carboxypeptidase B. However, studies by Proud and colleagues have since provided direct evidence of kinin generation during inflammatory reactions in humans. Bradykinin and lys-bradykinin are released into nasal lavage during experimentally-induced allergic rhinitis (Proud *et al.*,1983) and rhinovirus infections (Naclerio *et al.*, 1987). Such kinin generation was found to correlate with symptoms in both cases.

These observations raise the question as to whether or not kinins are mediators of the symptoms of allergic rhinitis. An association between kinin generation and nasal airway inflammation is not solely based on experimental models. Kinins appear in the nasal secretions of patients with seasonal allergic rhinitis (Svensson *et al.*, 1990) and during naturally occuring rhinovirus infections (Proud *et al.*, 1990). Further evidence for the involvement of kinins in allergic rhinitis is provided by the demonstration that application of bradykinin to the nasal airway induces an increase in nasal airway resistance and an increase in vascular permeability (Proud *et al.*, 1988; Rajakulasingam *et al.*, 1991).

The actions of bradykinin and lys-bradykinin are mediated via interaction with specific receptors. Two kinin receptor types have been described; the B_1 receptor and the B_2 type. The predominant receptor type in humans is the B_2 kinin receptor which is defined by its high affinity for bradykinin and low affinity for the carboxypeptidase metabolite, [Des-Arg^9]-bradykinin (Regoli & Barabe, 1980). The possible existance of a B_3 receptor has also been suggested (Farmer *et al.*, 1989). In the human nasal airway, a B_1 receptor agonist was found to be inactive in comparison with bradykinin and kallidin, and it was suggested that a B_2 receptor mediates the effects of bradykinin in the nasal airway (Rajakulasingam *et al.*, 1991).

The aim of the following experiments was to use antagonists to attempt to identify the kinin receptor involved in mediating the increase in nasal airway resistance and increase in vascular permeability induced by bradykinin in the human nasal airway.

4.2 Experimental protocol

For each experiment, subjects were first challenged with vehicle, then by active posterior rhinomanometry, 3 NAR measurements, each of 4 nasal cycles, were taken every 5 minutes over a fifteen minute period to provide a baseline.

On the first of two visits, subjects were given incremental doses of bradykinin, 10 to 1000μg to each nostril, at 30 min intervals. After each challenge, NAR was measured at 2,5,10,15 and 20 minutes.

In a second group of subjects, the effect of bradykinin and [Des-Arg^{10}]-kallidin was studied on the release of albumin into the nasal cavity. Subjects were given incremental doses of either bradykinin or [Des-Arg^{10}]-kallidin, 10 to 1000μg to each nostril at 30 minute intervals. Nasal lavage was performed 10 minutes after administration of each dose of kinin.

On a second occasion, subjects were given [Des-Arg^{10}]-kallidin, 100μg to each nostril and NAR was measured every 5 min for 20 min.

To study the action of the antagonists, another group of subjects were given the antagonist, 100μg to each nostril, 2 min prior to bradykinin, 100μg, and NAR was measured as before.

At a second visit, subjects underwent identical nasal treatments with the bradykinin antagonists and bradykinin, and a nasal lavage was performed 10 min after the challenge with bradykinin.

Data analysis

Changes in NAR in response to each substance were expressed as the percentage change from the vehicle control values. The response to each substance was quantified for each dose as the *maximal* change in NAR during the post-challenge measurements.

All data were expressed as mean $\pm$ s.e.m. Repeated measures analysis of variance (ANOVA) was used statistically to evaluate the responses to bradykinin alone. Student's t-test was used for the evaluation of the difference between values obtained after bradykinin challenge from those obtained after bradykinin challenge in the presence of the bradykinin antagonists. A probability value $p < 0.05$ was considered significant.

4.3 Results

4.3.1 Effect of bradykinin and [Des-Arg^{10}]-kallidin on nasal airway resistance and vascular permeability.

Figure 4.1 shows that bradykinin, 10 to 1000μg administered to each nostril, produced a dose-related increase in NAR (repeated measures ANOVA, p=0.016, n=8). Figure 4.2 demonstrates that over the same dose range, bradykinin also produced a dose-related increase in albumin content of nasal lavage (repeated measures ANOVA, p=0.02, n=10).

The selective B_1 kinin receptor agonist, [Des-Arg^{10}]-kallidin, 100μg was ineffective compared with bradykinin, 100μg in causing an increase in NAR (Figure 4.1) and did not affect the albumin content of nasal lavage (Figure 4.2).

4.3.2 Effect of three bradykinin antagonists on bradykinin-induced changes in the nasal airway.

Preliminary experiments showed that none of the bradykinin antagonists used in this study induced significant changes in NAR when administered alone, in the doses stated in the protocol.

The B_2 kinin receptor antagonist [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin, 100μg given 2 min prior to challenge with bradykinin, 100μg did not produce a significant reduction of the bradykinin-induced increase in NAR (figure 4.3 and 4.3a) or the albumin content of the nasal lavage (figure 4.4). Increasing the dose of this antagonist to 1000μg also failed to produce any reduction of the bradykinin-induced increase in NAR (n=5, p=0.277) (Figure 4.5).

The bradykinin antagonist [1-adamantane acetyl-D Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, 100μg given 2 min prior to challenge with bradykinin, 100μg produced a significant

(paired t-test, p=0.05, n=5) inhibition of NAR in response to bradykinin (figure 4.3 and 4.3b), and a corresponding inhibition (paired t-test, p=0.05, n=5) of the albumin content of the lavage (figure 4.4).

Figures 4.3, 4.3c and 4.4 show that the B_2 kinin receptor antagonist [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140), 100μg given 2 min prior to challenge with bradykinin, 100μg produced a significant inhibition of the bradykinin-induced increase in NAR (paired t-test, p=0.009, n=5) and of the albumin content of the lavage (paired t-test, p=0.037, n=5).

There was no significant difference (p=0.06, n=5) between the effectiveness of the active antagonists (BKA2 and BKA3).

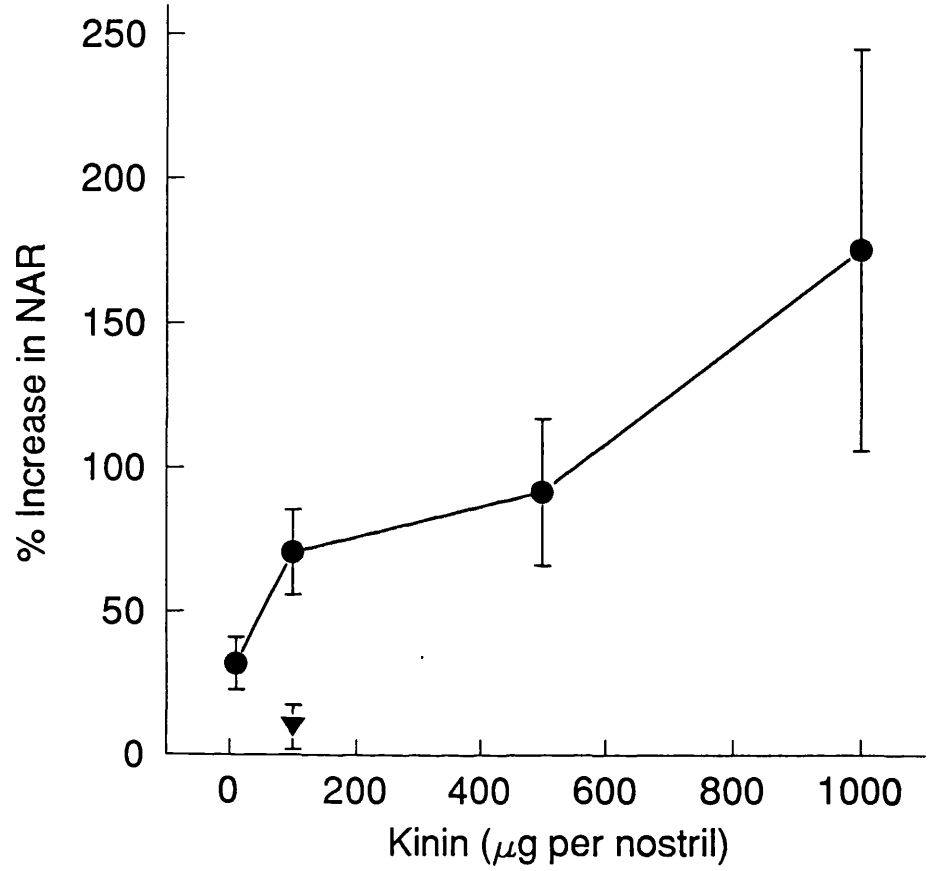


Figure 4.1.

Dose-response curve for bradykinin and increased nasal airway resistance (NAR). The effect of [Des-Arg^{10}]-kallidin (▼), 100μg is compared with bradykinin (●). The data are the means from 8 experiments for bradykinin and 5 experiments for [Des-Arg^{10}]-kallidin: vertical bars represent s.e.m. Baseline (saline) values for NAR were 0.320$\pm$0.019 Pa.s.cm^{-3} for bradykinin and 0.315 $\pm$ 0.014 Pa.s.cm^{3} for [Des-Arg^{10}]-kallidin.

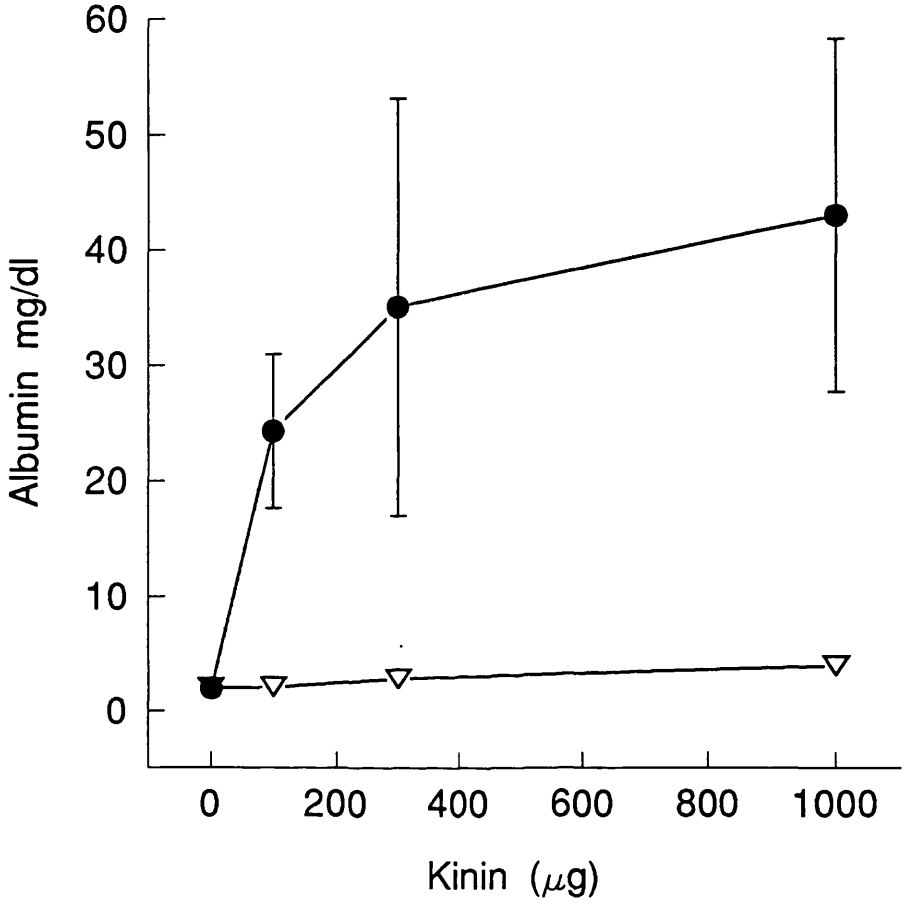


Figure 4.2

Dose-response curve for bradykinin and [Des-Arg^{10}]-kallidin, and albumin content of nasal lavage fluid.

(●) - bradykinin; (▽) - [Des-Arg^{10}]-kallidin. The data are the means from 5 to 10 experiments and vertical bars represent s.e.m. unless they are concealed within the symbol.

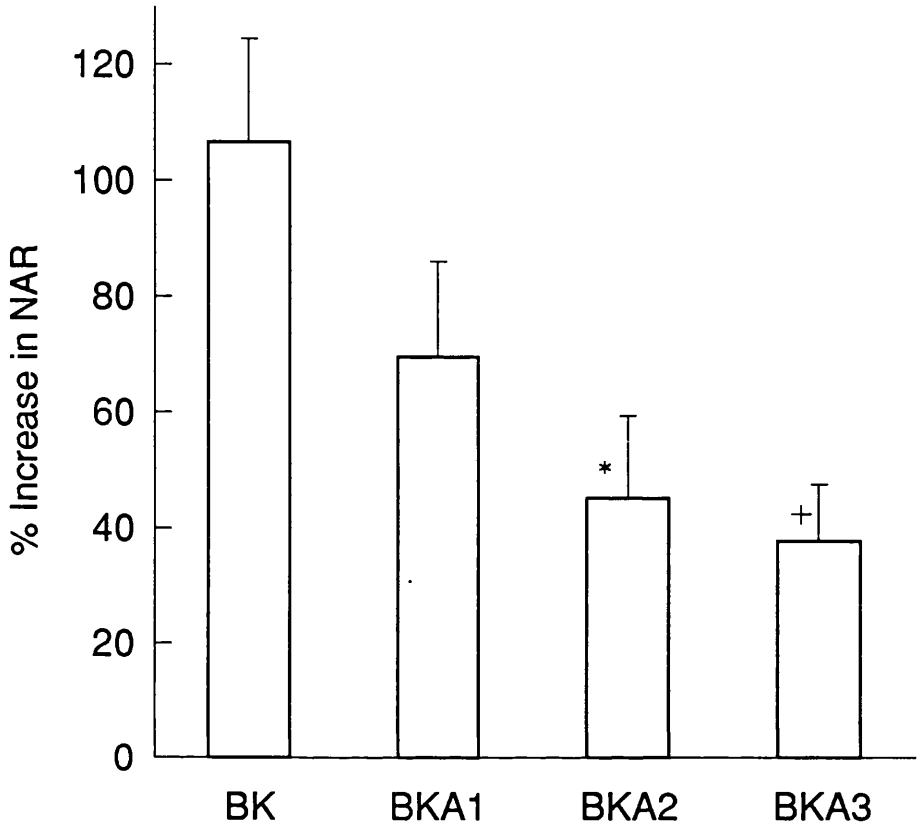
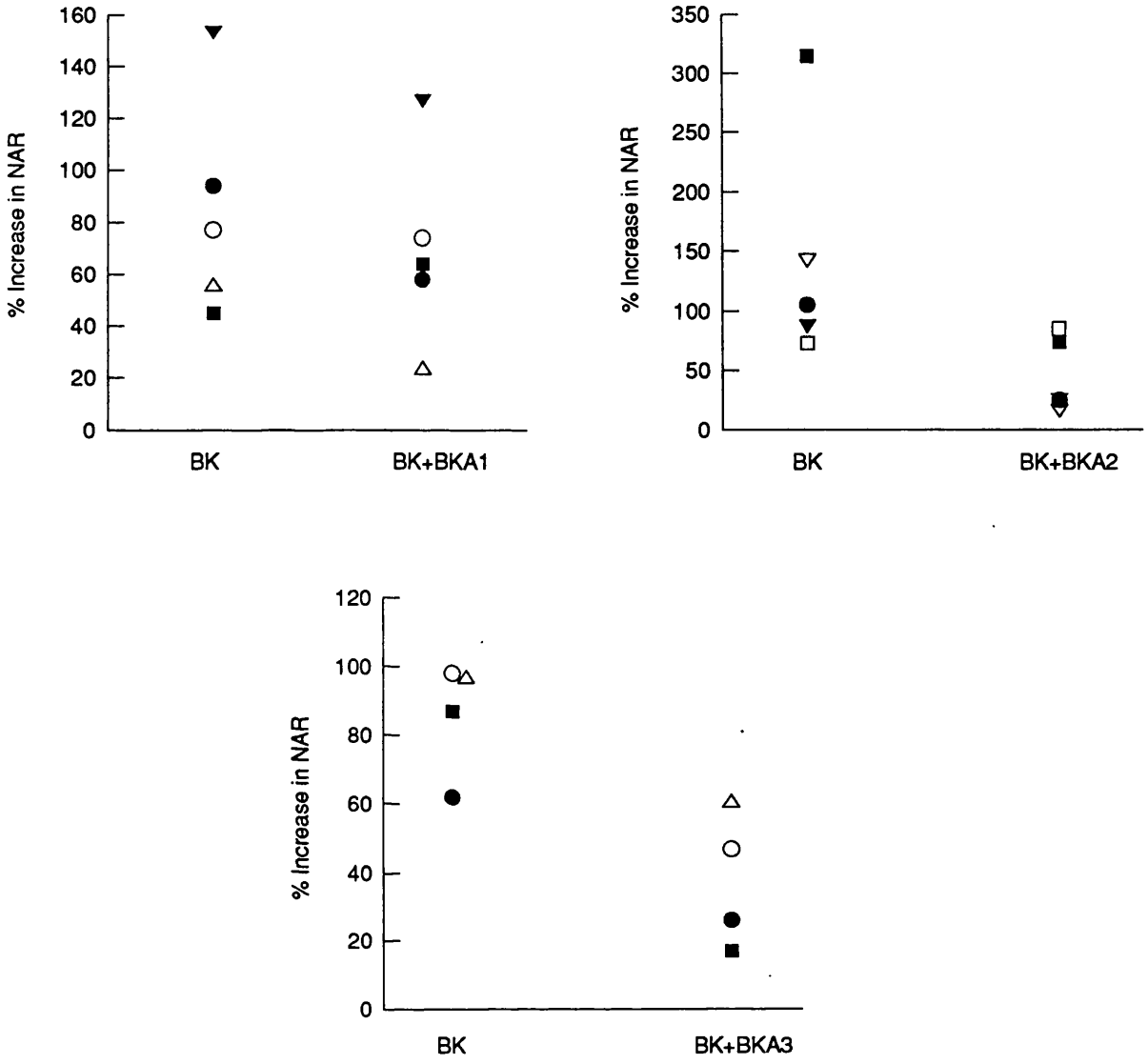


Figure 4.3

The effect of 3 BK antagonists on BK-induced increases in nasal airway resistance.

[D-Arg^0,Hyp^3,D-Phe^7]-bradykininis(BKA1),[1-adamantaneacetylD-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin is (BKA2) and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin is (BKA3). A dose of 100μg of each antagonist was given 2 min prior to challenge with bradykinin, 100μg. The responses are expressed as mean percentage increase in NAR and the data are the means from 5 experiments for each antagonist and 15 experiments for bradykinin alone. Vertical bars represent s.e.m. and *,+ indicate statistically significant differences (* p=0.05, + p=0.009). Baseline (saline) values for NAR were 0.350 $\pm$ 0.027 Pa.s.cm^3 for experiment with BKA1; 0.329 $\pm$ 0.020 Pa.s.cm^3 for BKA2; and 0.284 $\pm$ 0.024 Pa.s.cm^3 for BKA3.



Figures 4.3a, 4.3b and 4.3c

The same data as in figure 4.3 is represented showing individual changes in NAR following challenge.

[D-Arg^0,Hyp^3,D-Phe^7]-bradykininis(BKA1),[1-adamantaneacetylD-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin is (BKA2) and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin is (BKA3). A dose of 100μg of each antagonist was given 2 min prior to challenge with bradykinin, 100μg. The responses are expressed as percentage increases in NAR above control values. Each different symbol corresponds to a separate individual.

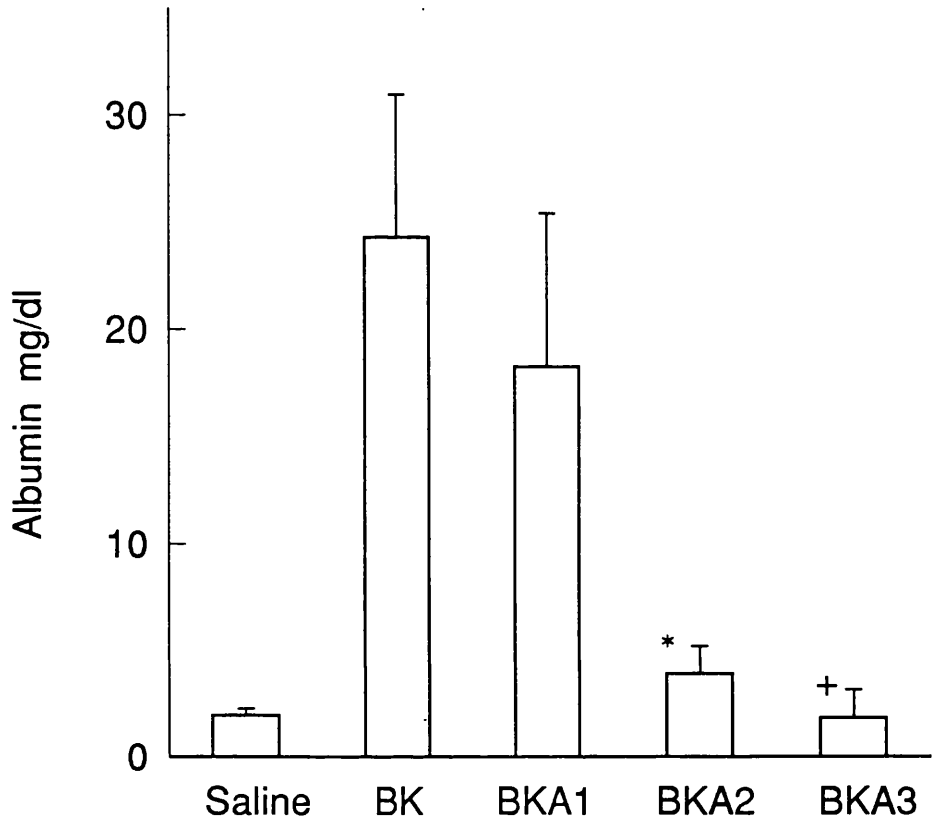


Figure 4.4

The effect of 3 BK antagonists on BK-induced increases in albumin content of nasal lavage.

[D-Arg^0,Hyp^3,D-Phe^7]-bradykinin is BKA1, [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin is BKA2 and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin is BKA3. For each antagonist, 100µg was given 2 min prior to bradykinin, 100µg and the effect on the bradykinin-induced increase in albumin content of nasal lavage fluid was measured. The data are the means from 5 experiments and vertical bars represent s.e.m. Statistically significant differences are indicated by *,+ (* p=0.05, + p=0.037).

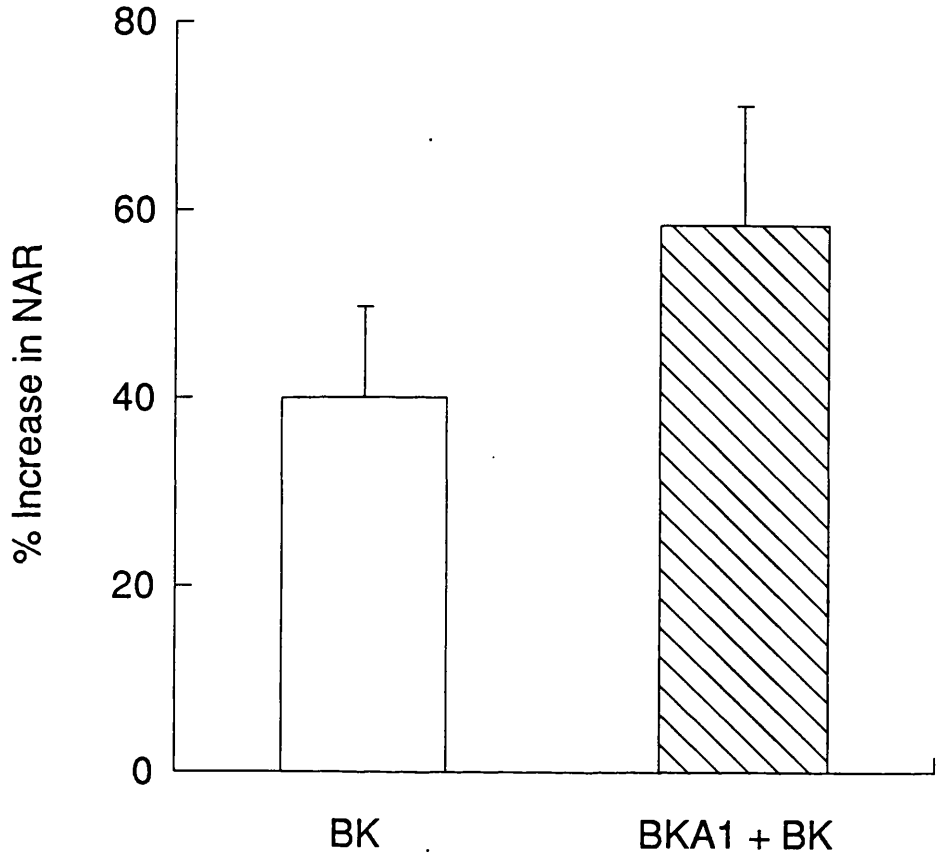


Figure 4.5

The effect of [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin (BKA1), 1000µg on the bradykinin-induced increase in nasal airway resistance.

The antagonist, 1000µg was given 2 min prior to bradykinin, 100µg. Responses are expressed as the mean percentage increase in NAR. The data are the means from 5 experiments for bradykinin alone (□) and 5 experiments for bradykinin in the presence of antagonist (▨). Vertical bars represent s.e.m. Baseline (saline) values for NAR were 0.225 ± 0.014 Pa.s.cm^3.

4.4 Discussion

The experiments presented in this chapter show that intranasally administered bradykinin induced a dose-related increase in NAR and an increase in albumin content of nasal lavage. Measurable effects were obtained with 10μg of bradykinin per nostril and a maximum effect does not appear to have been achieved, even with a dose of 1000μg. In this respect, the data is similar to that of others (Proud *et al.* 1988; Rajakulasingam *et* al. 1991), where doses between 20 and 2000μg, divided between the two nostrils, produced dose-related effects. The bradykinin-induced responses in the human nasal airway are likely to be the result of vasodilatation and microvascular leakage, mediated via activation of specific bradykinin receptors. There is some evidence that bradykinin may additionally stimulate sensory nerve endings, thus inducing the local release of other vasoactive peptides, such a substance P, neurokinin A and calcitonin gene-related peptide (Saria *et al.*, 1988). However Rajakulasingam *et al.*, (1992) have shown that although bradykinin-induced nasal discomfort appears to be neurally mediated, the effects of bradykinin on NAR and plasma protein exudation are not. The authors attributed these effects to a direct vascular action. In this study, no analysis of any indirect effects of bradykinin mediating the responses has been undertaken.

Kinin receptors have been classified into B_1 and B_2 types according to the relative potencies of a series of bradykinin analogues with agonist or antagonist properties (Regoli & Barabe, 1980; Regoli *et al.* 1990). More recently evidence has emerged indicating the possibility of a third type of kinin receptor, namely a B_3 receptor (Farmer *et al.* 1989; Farmer *et al.*, 1991b; 1991c; Field *et al.*, 1992). This is based on the observation that in some airways, neither B_1 or B_2 receptor antagonists have any effect on the contractile effects of bradykinin; neither is [Des-Arg^9]-bradykinin able to cause a response. Ligand binding studies corroborate these functional findings and Farmer *et al.*, (1989) demonstrated the presence of specific bradykinin binding in the guinea-pig trachealis which could not be displaced by a B_2 receptor antagonist. The authors suugested that the actions of bradykinin in this tissue may be mediated by a B_3 receptor. However, there are, as yet, no selective agonists or antagonists available for these receptors. It has also been suggested that there may be subtypes of the B_2 receptor; B_{2A} and B_{2B}. This is based

on evidence of differing affinities of B_2 antagonists in two different B_2 receptor-containing preparations (Rhaleb *et al.*, 1992). In the study of Rhaleb *et al.* (1992) [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin was equipotent on both preparations. There are several other reports of the existence of subtypes of B_2 receptor (Trifilieff *et al.*, 1991; Seguin *et al.*, 1992). In these studies, the authors claim that analysis of [^{3}H]-bradykinin binding kinetics suggests high and low-affinity binding sites, but that they were not sufficiently different to warrant the classification of a separate class of receptor (B_3).

In addition to quantifying the agonist effects of bradykinin in this study, the effect of a selective B_1 agonist in the nasal airway was also investigated. Removal of the N-terminal arginine from lys-bradykinin forms the selective B_1 agonist, [Des-Arg^{10}]-kallidin. Using this selective B_1 agonist in the present study, it caused no change in nasal airway resistance and there was no increase in vascular permeability, even at a dose of 1000μg. From this, it can be concluded that the kinin receptor mediating these nasal effects of bradykinin itself is not of the B_1 type. The same conclusion was also reached in another study using the selective B_1 agonist, [Des-Arg^9]-bradykinin (Rajakulasingam *et al.*, 1991). On the basis of the agonist potency order for B_2 receptors; bradykinin > kallidin > > > [Des-Arg^9]-bradykinin > [Des-Arg^{10}]-kallidin (Regoli & Barabe, 1980), it seems likely that the nasal effects of bradykinin are mediated through B_2 receptors. Bradykinin B_2 receptors have indeed been identified on vascular and sensory neural sites in the human nose, by ligand-binding studies using [^{125}I]-bradykinin (Baraniuk *et al.*, 1990c). Therefore, the bradykinin antagonists used in this study were selected on the basis of each displaying B_2 antagonist activity *in vitro*.

[D-Arg^0,Hyp^3,D-Phe^7]-bradykinin has been shown to be a competitive antagonist of B_2 receptors in several *in vitro* studies (Steranka *et al.*, 1989; Burch *et al.*, 1990) but the results of these studies and reports from others (Higgins *et al.*, 1990; Pongracic *et al.*, 1991) indicate that it fails to block the bradykinin-induced effects in the human nasal airway. This could result for several reasons. Although degradation of the antagonist cannot be ruled out, it seems unlikely that this explains its lack of effect in the human nasal airway. D-Phe^7 substituted analogues of bradykinin are resistant to the action of kininase II (Togo *et al.*, 1989), although they are susceptible to carboxypeptidase N

(Proud *et al.*, 1987). Moreover, if metabolism is a significant limitation to the effects of kinins in the nasal airway, then bradykinin itself would not be expected to be so active. More likely explanations are those related to the potency of the compound and the possibility that this antagonist is ineffective at the kinin receptor in question. [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin has a relatively low affinity for the B_2 receptor, having a pA_2 value on the order of 6 (Steranka *et al.*, 1989). This means that the dose initially used in this study, 100μg, may not have been sufficient. Therefore, the experiment was repeated using a dose of 1000μg, corresponding to a 10-fold molar excess, which was thought to be adequate to inhibit the effects of bradykinin in the nasal airway. However, such a dose was based on pA_2 values obtained in isolated animal tissue preparations. There is no data on *in vitro* testing of this compound on human cell or tissue preparations, and therefore it is possible that an even higher dose is required at the receptor level in man to achieve antagonism. Cost and ethical considerations precluded raising the dose any further. In an attempt to take account of the low potency and to favour occupancy of receptors by the antagonist, it was administered two minutes prior to bradykinin. Other studies where a 100-fold molar excess of the antagonist was used, still resulted in a total failure to induce inhibition of the effects of bradykinin (Pongracic *et al.*, 1991).

Recently, acylation of the N terminus of [D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin with 1-adamantane-acetic acid, has been shown to yield a highly potent bradykinin antagonist. This analogue has a ten-fold increased potency at B_2 receptors when compared with the parent compound (Lammek *et al.*, 1990), which is itself, a potent bradykinin anatagonist (Schacter *et al.*, 1987). [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin has been shown to antagonize the vasodepressor action of bradykinin in the rat and presumably acts at B_2 receptors (Lammek *et al.*, 1990), though an action on a different kinin receptor subtype has not been excluded. Some bradykinin antagonists acting on the vasculature, act as agonists at other sites, such as the adrenal where they stimulate the release of catecholamines. However, the acylation step in forming this antagonist appears to have eliminated activity which was a property of the parent compound (Lammek *et al.*, 1991). In the present study, this antagonist appears successfully to block the bradykinin-induced increase of NAR and vascular permeability in the human nasal airway. The high

potency of [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin has been attributed to the bulky substituent at the N-terminus, which is thought significantly to influence the interaction of the peptide with the B_2 receptor, thereby increasing potency (Lammek *et al.*, 1991).

Another novel, highly potent bradykinin antagonist, [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140) (Hock *et al.*, 1991; Wirth *et al.*, 1991), was found to inhibit the bradykinin-induced increase in NAR and albumin release into the nasal airway. This antagonist is highly potent, having an affinity for kinin receptors comparable with bradykinin. It is also long-acting and well-tolerated, since it has virtually no agonist activity (Hock *et al.*, 1991). The structure of this compound is such that it has a large hydrophobic region contributed by D-Tic^7,Oic^8 and this is thought to lead to prolonged occupation of, and greatly enhanced affinity for the bradykinin B_2 receptor (Rhaleb *et al.* 1992). It is also highly stable against enzymatic degradation, since it is not a substrate for kininase II or carboxypeptidases and is only slowly degraded in human plasma (Hock *et al.* 1991). *In vitro* and *in vivo* studies to date confirm that [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin has a high affinity for binding sites characteristic of B_2 receptors, having a pA_2 of 8.4 in guinea-pig ileum preparations (Hock *et al.*, 1991). However, it also labels a low-affinity binding sites in guinea-pig trachea (Trifilieff *et al.*, 1992) and shows agonist properties in this tissue, without exhibiting agonist effects in other B_2-receptor containing tissues (Hock *et al.*, 1991; Wirth *et al.*, 1991). This may be evidence for the B_3 putative receptor. Alternatively, kinins have indirect, receptor- independent effects and this may be an example.

The data presented in this chapter show that one antagonist, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin, which has previously been shown to act at B_2 receptors fails to inhibit the nasal actions of bradykinin that we have measured. In contrast, two other novel bradykinin antagonists do inhibit the nasal action of bradykinin. [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin is an antagonist of low potency and although we found it to be inactive even at a dose of 1000μg, we cannot exclude the possibility that it is active at the bradykinin receptor mediating increases in NAR. For the reasons discussed above, it seems unlikely that metabolism of the antagonist explains these effects but, again, this cannot

be excluded. Other factors such as conformational restraints, which may limit the access of the antagonist to the receptor site cannot be disregarded, although there is no information on this.

As pointed out above, there is published evidence which suggests the possibility that there is a B_3 receptor or that the B_2 class is subdivided. If it is accepted that [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin is inactive in the human nasal airway, the activity of the two other antagonists that we have demonstrated would be consistent with heterogeneity of B_2 receptors or the existence of a B_3 receptor.

The kinin receptor mediating increased NAR and increased vascular permeability in the human nasal airway is not B_1, and with the present pharmacological tools available, it can only be concluded that it appears to be B_2: whether it is 'B_3' or a subtype of the B_2 receptor requires more potent and selective antagonists and agonists.

Summary

- The aim of this study was to investigate the action of bradykinin on resistance to airflow and on vascular permeability in the human nasal airway of normal subjects, and to explore the receptor mediating these effects.

- Aerosol administration of bradykinin, 10 to 1000μg, caused a dose-related increase in nasal airway resistance (NAR), and an increase in albumin content of nasal lavage.

- The bradykinin antagonists, [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, 100μg, and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, 100μg, given 2 minutes before bradykinin, inhibited the increase in NAR and the increase of albumin content of nasal lavage caused by bradykinin.

- The bradykinin antagonist, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin, 100μg, did not affect the increase in NAR produced by bradykinin, or the albumin content of nasal lavage. Increasing the dose of the antagonist to 1000μg did not change the increase in NAR induced by bradykinin.

- The selective B_1 kinin receptor agonist, [Des-Arg^{10}]-kallidin, 100μg did not affect NAR or the albumin content of nasal lavage.

- The receptor mediating increased NAR and the release of albumin induced by bradykinin in the human nasal airway appears not to be a B_1 kinin receptor. The data are not entirely consistent with the effects of bradykinin in the human nasal airway being mediated by a B_2 kinin receptor.

CHAPTER 5

Pharmacological actions of bradykinin and bradykinin analogues in the human nasal airway. Part II: Atopic patients.

5.1 Introduction

Several recent reports have focused on demonstrating a possible role for kinins in certain pathological events in the airway. The previous chapter cited studies indicating a role of kinins in allergic reactions in the upper airway and during experimental and natural rhinovirus colds. Results from the studies performed in the previous chapter provide further support for the hypothesis that kinins may be important mediators in allergic rhinitis, since administration of bradykinin to the nasal airway causes symptoms of rhinitis, which can be prevented with a B_2 receptor antagonist. Definitive verification of this hypothesis can only be achieved by showing that blocking the actions of kinins during the nasal allergic reaction can lead to symptomatic relief.

In vivo studies in sheep (Soler *et al.*, 1990) have shown that inhalation of a B_2 receptor antagonist prior to antigen challenge inhibits the influx of neutrophils and hyperresponsiveness to methacholine, both of which are features of the allergic response in this model. Similarly, a B_2 receptor antagonist has been shown to be effective in inhibiting antigen-induced eosinophilia and hyperresponsiveness in a guinea-pig model (Farmer *et al.*, 1992). It was, therefore, one of the intentions of this study to investigate the effect of a B_2 receptor antagonist on the nasal allergic reaction.

The experimental results described in the previous chapter indicate that the acute effects of bradykinin challenge in normal individuals is probably mediated by the B_2 receptor type. This observation is consistent with reports from others (Churchill *et al.*, 1991; Rajakulasingam *et al.*, 1991). However, there is significant evidence that tissue injury or

inflammation could, by itself, modify the tissue responsiveness to kinins and, therefore, it is possible that the activity spectrum of kinins in atopic patients is somewhat different from that in normal individuals.

It has been shown *in vitro* and *in vivo,* that the B_1 receptor can be induced by inflammatory stimuli (Regoli *et al.*, 1978; 1981) and, therefore, the involvement of the B_1 receptor in inflammatory conditions such as allergic rhinitis, cannot be ruled out. If there were functionally significant B_1 receptors present in the inflammed nasal airway, then the normally inactive kinin fragments, [Des-Arg^9]-bradykinin and [Des-Arg^{10}]-kallidin, formed by the removal of the C-terminal arginine residue in the process of kinin metabolism, could act as vasodilators, and cause nasal blockage.

The aims of this study were two-fold. Firstly to investigate the effect of the B_2 antagonist, [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, on the nasal response to challenge with house-dust mite (HDM) in allergic patients; and secondly to investigate the possible functional importance of the B_1 receptor in the nasal airway of individuals with current symptoms of allergic rhinitis, in order to assess whether the B_1 receptor has a role in the generation of nasal symptoms associated with nasal allergy.

5.2 Experimental protocol

5.2.1 Effect of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin on HDM-induced increase in nasal blockage.

Ten patients took part in this experiment. They were treated by intranasal aerosol in a single blind, cross-over manner with: (a) saline (vehicle) or (b) [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, 200μg. Two minutes after the treatment, aerosol nasal challenge with HDM, 500U was performed. Patients were randomly allocated to either of the two treatments. The cross-over treatment was conducted one week later.

At each attendance, patients were first given an intranasal aerosol of sterile saline,

followed by acoustic reflection measurements performed every 5 minutes over a 15 minute period to collect baseline data. Symptom scores were also collected at this time. Patients were then given either treatment (a) or (b) as described above and acoustic reflection measurements and symptom scores were collected 10 minutes after challenge with HDM. Symptoms were recorded as nasal blockage, itching, sneezing and running, using 10cm visual analogue scales (Aitken, 1969).

5.2.2 Effect of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin on HDM-induced increase in vascular permeability:

For albumin measurements, 5 different patients took part in a single-blind, cross-over design similar to that described for acoustic reflection measurements. The two treatment groups were the same as described above for acoustic reflection measurements.

At each attendance, patients were given an intranasal aerosol of sterile saline and nasal lavage was performed 10 minutes later. Patients were then randomly allocated to treatments (a) or (b) and 10 minutes after challenge with HDM, 500U, nasal lavage was performed. One week later the cross-over measurements were made.

5.2.3 Effect of [Des-Arg^{10}]-kallidin on nasal blockage in atopic patients.

Five patients, all with current symptoms of allergic rhinitis and allergic to house-dust mite, took part in this study. Each patient was first given saline by aerosol and acoustic reflection measurements were made over a 15 minute period to collect baseline data. The patients underwent challenge with both [Des-Arg^{10}]-kallidin and bradykinin, separated by at least one hour. The study had a cross-over design as before, and patients received the challenge agents in a random order. Both kinins were administered by aerosol as a dose of 100μg to each nostril. Acoustic reflection measurements were made over 30 minutes following challenge. Patients recorded symptom scores on visual analogue scales (Aitken, 1969).

5.2.4 Data analysis

The mean of the A min. values obtained by acoustic rhinometry for the left and the right nostrils were calculated for each measurement taken. Responses were quantified as the maximal decrease in A min. during the post-challenge period, and expressed as % decrease from baseline (saline challenge). The total symptom score at each challenge was obtained by measuring the lines on the visual analogue scale and adding them. Albumin levels (mg/dl) in the lavage were calculated for saline and for each treatment.

All data were presented as mean $\pm$ standard error of mean. Student's t-test was used statistically to evaluate the differences between the responses to intranasal challenge with saline (baseline) and HDM and the responses to HDM and those obtained after HDM challenge in the presence of the bradykinin antagonist. One-way analysis of variance (ANOVA) was used to statistically evaluate the responses to [Des-Arg^{10}]-kallidin and bradykinin. A probability value of $p < 0.05$ was considered significant.

5.3 Results

5.3.1 Effect of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin on HDM-induced increase in nasal blockage.

Figures 5.1 and 5.2 show results from preliminary experiments designed to select the dose of house-dust mite (HDM) to be used in this study and the time at which to measure the responses. Figure 5.1 represents a dose-response curve to HDM, indicating that doses of 100 to 1000U to each nostril, induce dose-related decreases in A min. Figure 5.2 shows a time course of the response to house-dust mite, 500U in one subject.

Figure 5.3 shows that intranasal challenge with house-dust mite, 500U induced a decrease in A min. of 27.2 $\pm$ 5.8% ($p < 0.001$, $n = 10$), and an increase in symptom scores from control (saline) values of: 209% for nasal blockage; 51% for running; 32% for itching; 23% for sneezing (Figure 5.4). Only the magnitude of the change in nasal blockage

induced by HDM was significantly different from control scores ($p<0.05$). Administration of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, 200μg prior to challenge with house-dust mite, blocked the antigen-induced decrease in A min., ($p=0.006$, $n=10$) (Figure 5.3). In addition, the antagonist reduced symptom scores from those obtained after HDM challenge alone. However only the reduction in scores for nasal blockage reached statistical significance ($p<0.05$; Figure 5.4).

5.3.2 Effect of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin on HDM-induced increase in vascular permeability:

Nasal challenge with HDM, 500U had no effect on the concentration of albumin measured in nasal lavage. Albumin levels after HDM were no different from control (saline) levels. The mean albumin concentration of nasal lavage prior to challenge with HDM was 3.8 $\pm$ 0.6 mg/dl and after HDM challenge, it was 2.9 $\pm$ 0.8 mg/dl ($n=5$, $p>0.05$).

5.3.3 Effect of [Des-Arg^{10}]-kallidin on nasal blockage in atopic patients.

Figure 5.5 shows that administration of the selective B_1 agonist, [Des-Arg^{10}]-kallidin, 100μg to atopic patients presenting with current symptoms of allergic rhinitis had no effect on nasal blockage, as quantified by no significant change in A min. values (ANOVA $p>0.05$). In contrast, Figure 5.5 shows that bradykinin, 100μg caused a significant decrease in A min. (ANOVA $p<0.0001$).

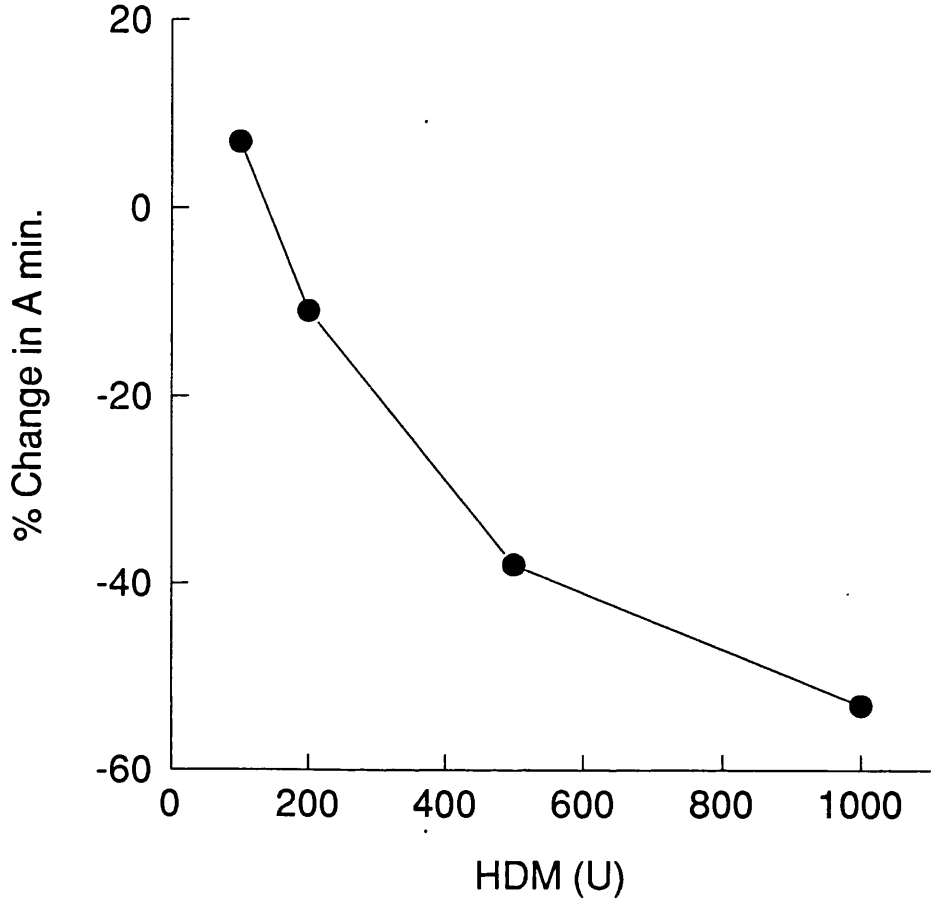


Figure 5.1

Dose-response curve for nasal challenge with house-dust mite.

Responses to challenge with house-dust mite (HDM) in one patient, expressed as the maximal percentage change from control in the minimum nasal cross-sectional area (A min.) during the post-challenge period. Control (saline) value for A min. was 0.44cm^2.

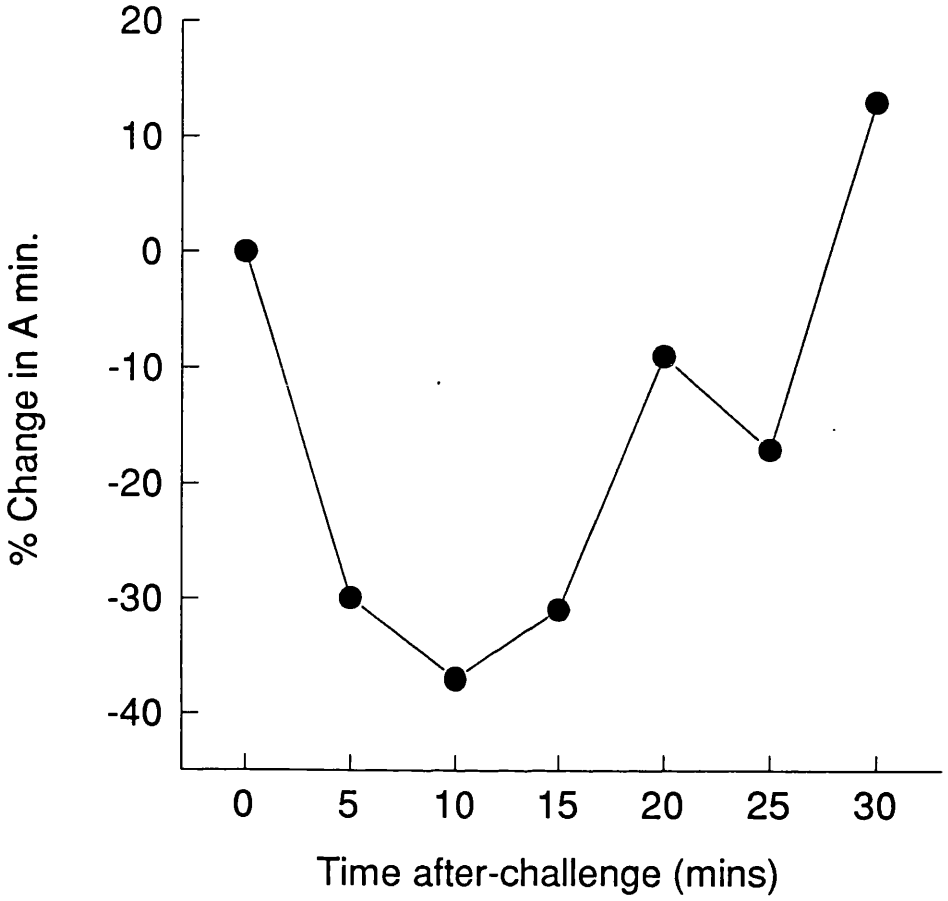


Figure 5.2.

Time course of the response to challenge with house-dust mite.

Example of the response to HDM, 500U with time, in one atopic patient. Responses are expressed as the percentage change in the minimum nasal cross-sectional area (A min.) from the vehicle (saline) control value, which was 0.23cm^2.

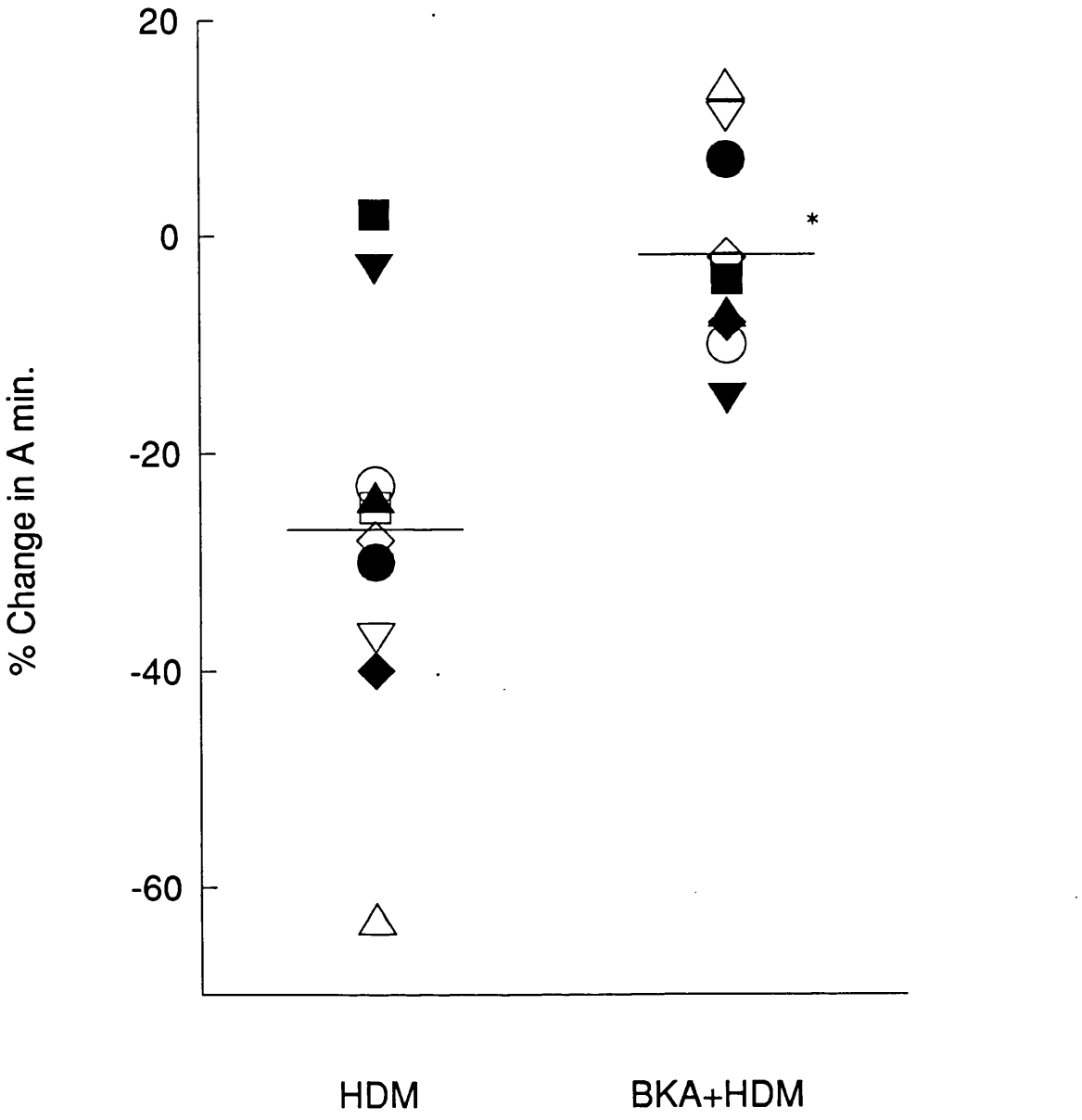


Figure 5.3

The effect of a bradykinin antagonist on allergen-induced increases in nasal blockage.

[D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (BKA) 200µg, or vehicle were given 2 min prior to challenge with house-dust mite, 500U. The responses to intranasal allergen challenge are expressed as % decrease in the minimum nasal cross-sectional area (A min.). Each symbol represents a different individual. The horizontal bars represent the means from the 10 experiments. Control (saline) values for A min. were 0.41 ± 0.05cm^2 for challenge with house-dust mite, and 0.43 ± 0.06cm^2 for house-dust mite challenge in the presence of antagonist. * - significant difference, $p<0.05$.

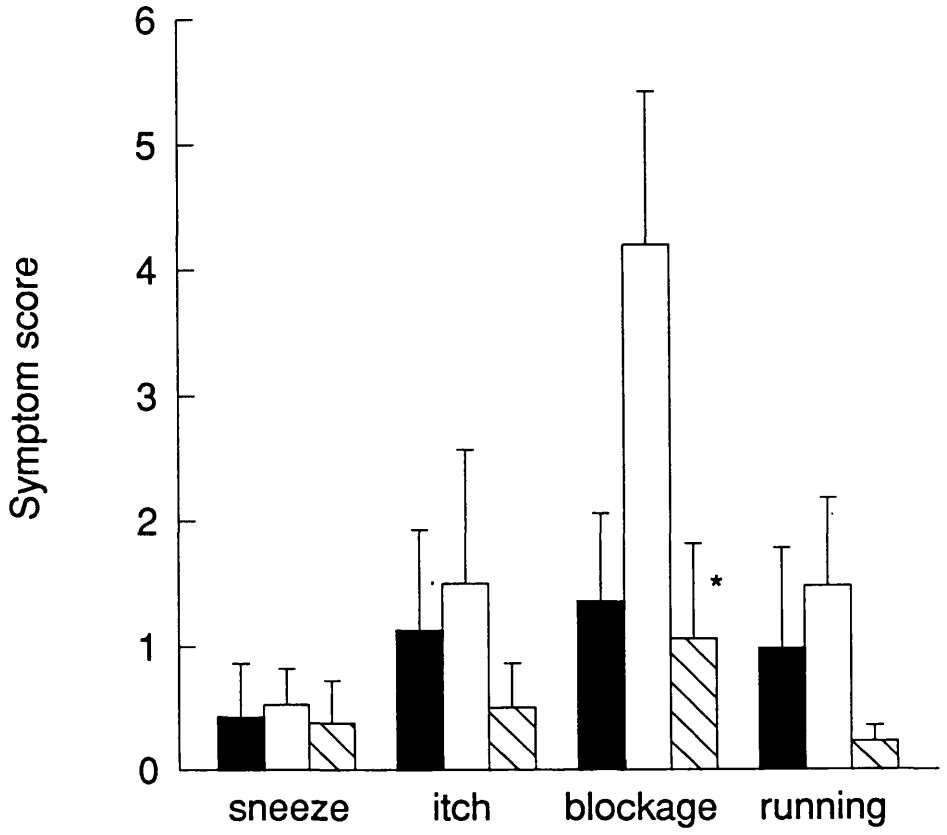


Figure 5.4.

The effect of a bradykinin antagonist on allergen-induced increases in symptom scores.

[D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (BKA) 200μg, or vehicle was given 2 min prior to house-dust mite, 500U. (■)- saline challenge; (□) house-dust mite challenge; (▨) house-dust mite challenge in the presence of bradykinin antagonist, 200μg. The data are the means from 10 experiments and vertical error bars represent s.e.m. * - significant difference, $p < 0.05$.

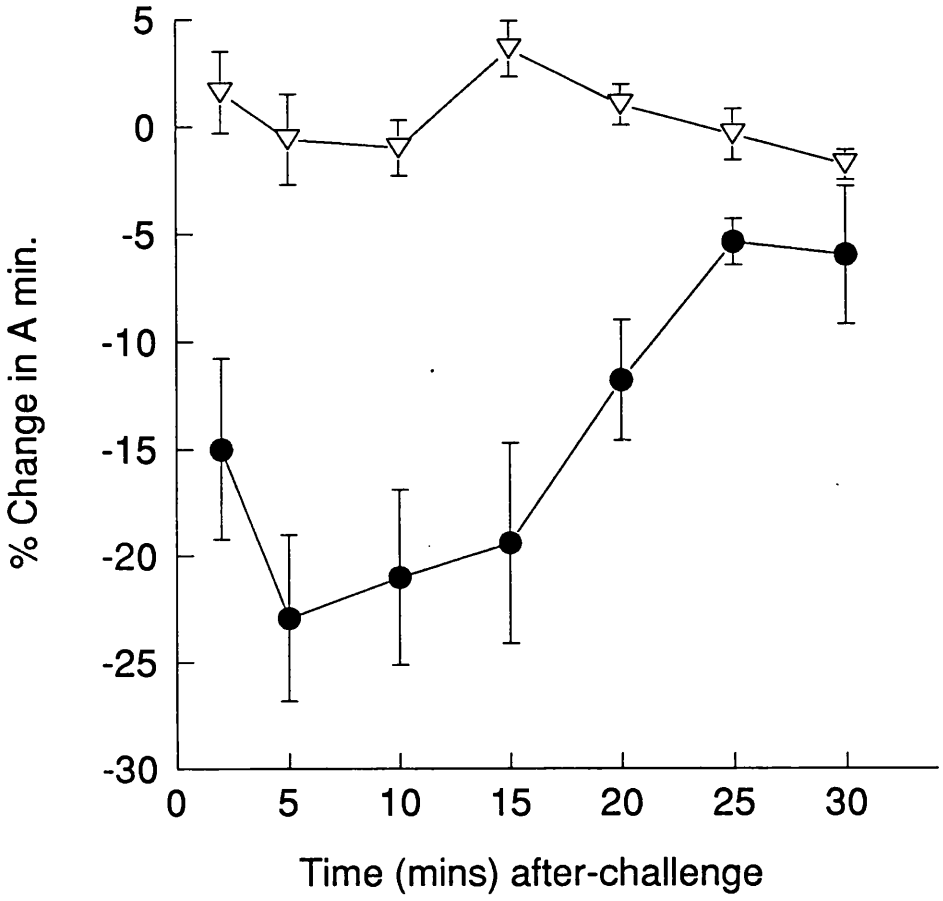


Figure 5.5.

The effect of the B_1 receptor agonist, [Des-Arg^{10}]-kallidin compared with bradykinin on nasal blockage in rhinitic patients.

[Des-Arg^{10}]-kallidin, 100μg (▽) and bradykinin, 100μg (●)were administered to 5 individuals. Responses are expressed as the percentage change in minimum nasal cross-sectional area (A min.). Vertical bars represent s.e.m. Control (saline) values for A min. were 0.397 $\pm$ 0.06cm^{2}prior to [Des-Arg^{10}]-kallidin challenge and 0.413 $\pm$ 0.03cm^2 prior to bradykinin challenge.

5.4 Discussion

This study identifies a kinin-mediated component in the response to nasal challenge with house-dust mite (HDM) in individuals with allergic rhinitis to this antigen. The significant inhibition of the HDM-induced increase in nasal blockage by this antagonist and the lack of effect of HDM on vascular permeability, indicates that the kinin-mediated component of this response, consists predominantly of nasal blockage. This is supported by the symptom scores recorded. The antagonist reduced all symptoms; nasal blockage, rhinorrhoea, itching and sneezing, but only in the case of nasal blockage, was the reduction statistically significant. These data confirm those of many others where a potential role for kinins in nasal inflammatory and allergic reactions has been suggested.

The effect of the bradykinin antagonist on the nasal response to HDM challenge was examined in this study because bradykinin, itself, induces changes in nasal blockage and vascular permeability (Proud *et al.*, 1988; chapter 4), both of which have been reported to occur in nasal allergic reactions (Proud *et al.*, 1983; Baumgarten *et al.*, 1985). The antagonist used in this study was selected on the basis of it being the most effective compound used in the previous study, to block the effects of exogenous bradykinin. The dose of antagonist used was partially based on the previous study, although it was increased, because the amount of endogenous kinin produced by each patient following allergen challenge was unknown. Kinin generation in the nasal airway has previously been measured in allergic patients following allergen challenge (Proud *et al.*, 1983), but a wide variation was evident, making it impossible to predict the likely concentrations in the nasal secretions of patients in this study.

The dose of HDM used for the allergen challenge in this study was based on preliminary experiments, which indicated that a dose of 500U produced a sub-maximal response in nasal blockage, i.e. sub-maximal decrease in A min. values, in all patients studied. In the preliminary experiments, the time course of the HDM-induced increase in nasal blockage was also investigated. It was found that the response was maximal at approximately 10 minutes post-challenge. Therefore, in the present study the HDM-induced effects on nasal blockage and vascular permeability, were quantified at 10 minutes post-challenge.

The results of these experiments demonstrate that HDM induced an increase in nasal blockage, which could be blocked with, [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, but that HDM had no effect on vascular permeability, as indicated by measuring the albumin concentration of nasal lavage. Nasal challenge with grass pollen has previously been shown to induce increases in vascular permeability at 40 minutes post-challenge, a time which correlated with the onset of symptoms (Baumgarten *et al.*, 1985). In this study, vascular permeability was examined at 10 minutes post-challenge, since this time corresponded to the maximal effect of the allergen on symptoms, as determined by quantifying nasal blockage.

A previous study investigating the effect of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin in nasal allergy, indicated that it was ineffective in reducing the response (Akbary & Bender, 1993). However, this study was performed using seasonal allergic rhinitics, with allergy to grass pollen. The difference between the two studies is interesting, because patients with perennial allergic rhinitis, with chronic HDM sensitivity, suffer predominantly from nasal blockage, whereas those with seasonal allergic rhinitis, with grass pollen sensitivity, have marked rhinorrhoea and sneezing (Dr G.K. Scadding, The Royal National Throat, Nose and Ear Hospital, London, unpublished observations). The pathophysiology of the two types of rhinitis may differ, with bradykinin being a major mediator of perennial rhinitis but not seasonal allergic rhinitis. Despite the fact that exogenous bradykinin can induce increases in both nasal blockage and nasal vascular permeability (Proud *et al.*, 1988; chapter 4), it may be that antigen provocation, in contrast, causes more local increases in kinins within the nasal airway, resulting in increased blood flow into nasal sinusoids. This may be sufficient to cause nasal blockage via soft tissue swelling and may be more important than blockage induced by swelling caused by plasma extravasation. However, another possibility which cannot be ruled out at the present time, is that the dose-response curve for HDM and vascular permeability may be shifted, in comparison with that for HDM and nasal blockage. This seems unlikely though, given the clinical observations and lack of effect of the antagonist in seasonal allergic rhinitis.

The data in this chapter provide the first demonstration of a bradykinin antagonist

producing an effective blockade of allergen-induced symptoms in a human model of allergic rhinitis. The bradykinin component of the response to house-dust mite challenge appeared to consist largely, of nasal blockage. Bradykinin has a direct vasodilatory effect causing engorgement of vessels limiting airflow, (Regoli & Barabe, 1980). It also has an indirect effect, by virtue of an ability to sensitize and stimulate, capsaicin-sensitive sensory C-fibres (Saria *et al.*, 1988). This leads to the release of neuropeptides, probably via axon reflexes, resulting in vasodilatation and gland secretion, which could be postulated to cause nasal blockage and oedema respectively. However, a more recent study where subjects were challenged with intranasal bradykinin, indicated that only bradykinin-induced rhinorrhoea is neurally mediated, and the effects on nasal blockage are due to the direct vascular action of this peptide (Rajakulasingam *et al.*, 1992b).

The rhinitic effects of bradykinin have been reported previously (Proud *et al.*, 1988; Rajakulasingam *et al.*, 1991). They have been attributed to activation of B_2 receptors (Rajakulasingam *et al.*, 1991; chapter 4), which have been shown, by autoradiographic examination, to be present on the nasal vasculature (Baraniuk *et al.*, 1990c). However, previous studies which have investigated the effect of a B_2 receptor antagonist in the nasal airway, either after bradykinin challenge or during viral rhinitis, have not been encouraging. These studies, (Higgins *et al.*, 1990; Pongracic *et al.*, 1991) which are discussed in the previous chapter, employed [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin as the antagonist. The lack of success with this antagonist is probably associated with the lack of potency or specificity of the compound. Therefore the use of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, a new, more potent and more stable antagonist (Hock *et al.*, 1991; Wirth *et al.*, 1991) was necessary, in order to examine further the possible kinin contribution in the nasal allergic response.

The effect of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin on attenuating the nasal response to allergen challenge was striking. Given the magnitude of the effect, it is possible that the action of this bradykinin antagonist is not simply at the level of blocking the effects of endogenous kinin *per se*. In addition to receptor-mediated effects, the possible contribution of receptor independent effects should be considered. Bradykinin and its peptide analogues are known to exhibit several non receptor-mediated effects. Of

relevance here, is that some antagonists have been reported to inhibit human tissue kallikrein (Spragg *et al.*, 1988). Such inhibition would lead to a reduction in the conversion of kininogen to kinin at multiple sites in the body. It is, therefore, possible that the presence of a bradykinin antagonist in the nasal airway, could result in a local reduction in kinin generation. Spragg *et al.* (1988) monitored this effect *in vitro* and found that it occured within 10 minutes, which is appropriate to the time course of the nasal response.

The effectiveness of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin in attenuating the nasal response to allergen challenge in patients with allergy to house-dust mite, is evidence for bradykinin involvement in this response, which is B_2 receptor mediated, according to current bradykinin receptor classification (Regoli *et al.*, 1990). The possibility that B_1 receptors may be present in the nasal airway of allergic patients and that they may have a role in producing rhinitic effects, was also investigated. This was based on studies which have implicated B_1 receptors pathophysiologically in tissues which are normally only responsive to B_2 agonists. The first report indicating that inflammed or injured tissue may differ from normal tissue in responsiveness to kinins, was provided by Regoli *et al.* (1978). It was shown that the response of isolated rabbit mesenteric vein to the selective B_1 receptor agonist, [Des-Arg^9]-bradykinin, consistently increased, from an initial low level, as a function of incubation time *in vitro*. A similar phenomenon has been confirmed in other isolated tissues including human colon (Couture *et al.*, 1981). The upregulated response to [Des-Arg^9]-bradykinin is believed to be due to the *de novo* synthesis of B_1 receptors during incubation. This is supported by the ability of cycloheximide and actinomycin, which inhibit protein synthesis and RNA synthesis respectively, but not anti-inflammatory agents, to ablate the spontaneously generated responsiveness (Regoli *et al.*, 1978). Ligand binding studies have shown a correlation between an increase in specific binding of [^{3}H]-des-Arg^9-bradykinin with its time dependent ability to cause a functional response (Barabe *et al.*, 1982). Following these *in vitro* experiments, responsiveness to [Des-Arg^9]-bradykinin has been induced *in vivo* at the site of chemical trauma, and blocked with Des-Arg^{10},[Leu^9]-kallidin, a B_1 antagonist (Marceau *et al.*, 1980; Regoli *et al.*, 1981).

Therefore, this study examined whether a pharmacological response to the B_1 agonist, [Des-Arg^{10}]-kallidin, could be achieved in patients with allergic rhinitis. It was found that [Des-Arg^{10}]-kallidin had no effect in the nasal airway, whereas bradykinin challenge in these patients induced a significant increase in nasal blockage. Such an effect of bradykinin is further evidence for the B_2 receptor being involved in the nasal response to allergen challenge, but suggests that there is unlikely to be a contribution from the B_1 receptor. This data cannot be taken as conclusive evidence for this, however, since the induction of B_1 responsiveness may occur after an actual insult, such as an allergen challenge. Even though these patients all had current symptoms of perennial rhinitis, they were not of the same magnitude as those symptoms observed directly after an allergen challenge. The studies described above on induction of B_1 responsiveness provided evidence for this phenomenon being maximal *in vitro* at 6 hours (Regoli *et al.*, 1978) and *in vivo* at 5 hours after injury (Marceau *et al.*, 1980; Regoli *et al.*, 1981). It may, therefore, be more appropriate to perform an allergen challenge and investigate responsiveness to a B_1 agonist over the post challenge period. In the light of the studies described above on induction of B_1 responsiveness, the effect may occur several hours after a challenge and the B_1 responses may return to a null level outside a challenge. However, studies which have investigated responses to B_1 receptor agonists in the nasal airway over 6 hours after challenge found no evidence for induction of B_1 responsiveness (Dr D. Proud, Johns Hopkins University School of Medicine, Baltimore, U.S.A. - personal communication).

If B_1 receptors have any role in allergic rhinitis, the mechanism by which tissue injury may promote *de novo* formation is unknown. Such B_1 receptor generation would have no consequence if the kinin metabolites, [Des-Arg^9]-bradykinin and [Des-Arg^{10}]-kallidin, derived from kininase I were not present at the site of injury. It is unknown whether the stimulus for B_1 formation has any effect on kininase I. *In vivo*, kininase II is largely responsible for kinin inactivation (Marceau *et al.*, 1983). Again, whether kininase I predominates in certain pathological conditions remains unknown. Kininase I being a plasma protein may be present in inflammatory exudate and might promote the production of B_1 agonists at tissue level. [Des-Arg^9]-bradykinin has a greater capacity to induce effects than bradykinin itself, since it possesses a significantly longer half-life in plasma

(Marceau *et al.*, 1981).

The results from the present study provide no evidence for B_1 receptor-mediated effects in the allergic nasal airway. It would appear that kinin-mediated effects in the nasal airway, relevant to allergic rhinitis occur via a B_2 receptor, since [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin is very effective in attenuating the nasal blockage induced by HDM, in patients with this allergy. However, it seems that the relative contribution of inflammatory mediators in perennial and seasonal allergic rhinitis may differ. The bradykinin antagonist used in this study may be useful in further studies, to clarify this point. At present, it can only be concluded that bradykinin antagonists may have a potential therapeutic application, at least in some types of allergic rhinitis.

SUMMARY

- The effect of the bradykinin antagonist, [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin on the response to nasal challenge with house-dust mite (HDM) in 15 atopic patients has been investigated. Responses were assessed using acoustic rhinometry, by measuring changes in vascular permeability, and by recording symptoms on visual analogue scales.

- Intranasal challenge with HDM, 500U resulted in significant increases in nasal blockage but had no effect on vascular permeability.

- Administration of [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin, 200μg, 2 min prior to challenge with HDM, 500U significantly reduced the HDM-induced nasal blockage. The data indicate that the nasal response to allergen challenge is mediated to a large degree by kinins.

- The selective B_1 receptor agonist, [Des-Arg^{10}]-kallidin, has no effect on nasal blockage or any symptoms scored. This is in contrast to bradykinin, which increased nasal blockage and symptom scores.

CHAPTER 6

The contribution of histamine to the action of bradykinin in the human nasal airway.

6.1 Introduction

One of the first reports indicating that kinins may be involved in the nasal allergic response demonstrated that nasal challenge of allergic individuals with allergen, results in the appearance of kinins in nasal lavage fluid (Proud *et al.*, 1983). Such kinin generation was shown to correlate highly with the onset of symptoms and with presence of other inflammatory mediators, particularly those derived from mast cells, such as histamine and PGD_2. It has since been established that intranasal administration of bradykinin results in rhinorrhoea and nasal blockage (Proud *et al.*, 1988; Rajakulasingam *et al.*, 1991; chapter 4). These effects are in keeping with the vasodilator effect of bradykinin and its effect on increasing vascular permeability, and have been attributed to a direct, receptor-mediated action, probably involving the kinin, B_2 receptor (Rajakulasingam *et al.*, 1991; chapter 4). However, the precise mechanism has yet to be clarified and in addition, kinins may also have indirect, kinin receptor-independent effects. Receptor-independent mechanisms have been proposed for kinins in guinea-pig trachea (Mizrahi *et al.*, 1982) and rat peritoneal mast cells (Devillier *et al.*, 1989).

It has previously been shown that the effects of bradykinin in the nasal airway are not dependent on prostanoid generation (Churchill *et al.*, 1991), neither are they influenced by the selective antimuscarinic agent, ipratropium bromide (Rajakulasingam *et al.*, 1992). However, rodent studies suggest that mast cell degranulation may contribute to the effects of bradykinin (Ishizaka *et al.*, 1985) and it has also been shown that analogues of bradykinin can act as histamine secretagogues in human skin mast cells (Lawrence *et al.*, 1989). In view of these findings and the above mentioned correlation between kinins, histamine and PGD_2, following allergen challenge, the following studies were designed

to investigate the contribution of histamine to effects of intranasal bradykinin challenge, by using selective H_1-antagonists and by measuring histamine release following kinin challenge.

6.2 Experimental protocol

6.2.1 Protocol for nasal challenge experiments

For each experiment, subjects were first challenged with vehicle solution, then by active posterior rhinomanometry, 3 NAR measurements were taken every 5 minutes over a fifteen minute period. This formed the baseline.

In the initial study subjects were given incremental doses of bradykinin, 10 to 1000μg to each nostril at 20 minute intervals, both with and without the presence of cetirizine (10mg) or terfenadine (60mg), given orally 3 hours before. In a further study, subjects were given 100μg bradykinin, and 30 minutes later, 100ug of [1-adamantaneacetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, 2 minutes prior to a repeat 100μg dose of bradykinin. This, as before, was done both with and without pretreatment with cetirizine (10mg, given 3 hours before). A study using histamine and [1-adamantaneacetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin was also done using the same timings.

After each challenge, NAR was measured at 2, 5, 10, 15 and 20 minutes and subjects recorded their nasal symptoms on score sheets during each challenge period. A total symptom score was obtained from individual symptom ratings as detailed: for nasal blockage, 0=not blocked, 1=slightly blocked, 2=moderately blocked, 3=severely blocked: for rhinorrhoea, 0=absent, 1=present, 2=severe: for sneezing, 0=no sneezes, 1=<4 sneezes, 2=>4 sneezes: for pain, 0=absent, 1=slight pain, 2=severe pain: for itching, 0=absent, 1=present.

In a second study, subjects were challenged with vehicle (saline) followed by bradykinin, 100 to 1000μg to each nostril. Ten minutes later, a nasal lavage was performed and the

albumin concentration of the nasal lavage was determined. This was repeated in the presence of cetirizine, 10mg taken 3 hr before bradykinin challenge.

The histamine concentration of nasal lavage was also determined after bradykinin challenge. Subjects were given saline, then 300 or 1000μg bradykinin and underwent a nasal lavage 10 minutes after each challenge.

6.2.2 Protocol for histamine release experiments

Histamine release from rat peritoneal mast cells was quantified in response to 10 minute incubations with; bradykinin, 10 to 100μM; [Des-Arg^{10}]-kallidin, 10 to 100μM; and bradykinin, 10 and 30μM in the presence of [D-Arg^{0},Hyp^{3},D-Phe^{7}]-bradykinin (BKA1), 10 and 30μM, [1-adamantane acetyl-D-Arg^{0},Hyp^{3},Thi^{5,8},D-Phe^{7}]-bradykinin (BKA2), 10 and 30μM and [D-Arg^{0},Hyp^{3},Thi^{5},D-Tic^{7},Oic^{8}]-bradykinin (BKA3), 10 and 30μM.

6.2.3 Data analysis

Changes in NAR in response to each substance were expressed as the percentage change from the saline baseline values. The response to each substance was quantified for each dose as the maximal change in NAR during the post-challenge measurements. Histamine release from rat peritoneal mast cells was expressed as a percentage above basal release.

All data was expressed as mean $\pm$ s.e.m. Repeated measures analysis of variance (ANOVA) was used statistically to evaluate the responses to incremental doses of bradykinin, both in the presence and absence of H_1-receptor antagonists. A probability value of $p<0.05$ was considered significant. Student's t-test was used to statistically evaluate some of the data where appropriate and as indicated.

6.3 Results

6.3.1 The effect of H_1- receptor antagonists on the response to nasal bradykinin challenge.

Bradykinin, 10 to 1000μg in each nostril produced a dose-related increase in NAR (Figure 6.1, repeated measures ANOVA, $p=0.016$, $n=8$). The histamine H_1-antagonists, cetirizine, 10mg orally ($n=5$), and terfenadine, 60mg orally ($n=6$) both inhibited the increase in NAR induced by bradykinin (Figure 6.1, repeated mesures ANOVA, $p>0.05$ for both). Over the same dose range, bradykinin also produced a dose-related increase in total symptom scores in the subjects (Figure 6.2). Terfenadine, 60mg orally did not suppress the total symptom score in response to bradykinin and cetirizine, 10mg orally only produced a statistically significant reduction of total symptom score for the highest dose of bradykinin (Figure 6.2). Increasing the dose of cetirizine from 10mg to 20mg produced no greater effect on NAR (Figure 6.3).

To study the role of histamine on the responses to lower doses of bradykinin, the lower region of the dose-response curve was specifically investigated. Figure 6.4 shows that the bradykinin-induced increases in NAR, 10 to 100μg in each nostril, (repeated measures ANOVA, $p=0.003$, $n=5$) were attenuated by pretreatment with cetirizine, 10mg orally, but this did not reach statistical significance. There was a dose-related effect of bradykinin remaining (repeated measures ANOVA, $p=0.035$, $n=6$).

6.3.2 The effect of a bradykinin antagonist in the presence of an H_1-receptor antagonist on the response to nasal bradykinin challenge.

The bradykinin antagonist, [1-adamantaneacetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, 100μg given 2 min prior to challenge with bradykinin, 100μg produced a significant inhibition of NAR in response to bradykinin (Figure 6.5; $n=5$). The antagonist itself produced no significant change in NAR. In the presence of cetirizine, the bradykinin antagonist produced a further decrease in NAR (Figure 6.5; $n=4$) and [1-

adamantaneacetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, 100μg did not significantly antagonize the action of histamine, 200μg on NAR (Figure 6.6; n=5).

6.3.3 The effect of an H_1-receptor antagonist on bradykinin-induced increases in vascular permeability.

Bradykinin 100 to 1000μg produced a dose-related increase in vascular permeability as quantified by albumin release in nasal lavage (n=10; Figure 6.7). Pretreatment with cetirizine, 10mg, 3 h prior to bradykinin challenge resulted in a decrease of albumin release into nasal lavage. This reached statistical significance at bradykinin, 1000μg (Figure 6.7; n=8; p=0.108).

6.3.4. The effect of bradykinin challenge on the histamine concentration of nasal lavage.

Bradykinin, 300μg (n=15) and 1000μg (n=32) had no effect on the histamine concentration of nasal lavage taken 10 minutes following the challenge, as compared with the histamine concentration of nasal lavage after saline challenge (n=47) (Table 6.1).

6.3.5. The effect of bradykinin and bradykinin analogues on histamine release from rat peritoneal mast cells *in vitro*.

Incubation of rat peritoneal mast cells with bradykinin, 10 to 100μM and [Des-Arg^{10}]-kallidin, 10 to 100μM resulted in dose-related increases in histamine release (Figure 6.8; n=5 to 10 experiments for each; repeated measures ANOVA for BK, $p<0.0001$ and for [Des-Arg^{10}]-KD, $p<0.0001$). Bradykinin was approximately 1.6 times more potent, in terms of histamine releasing activity, than [Des-Arg^{10}]-kallidin.

The bradykinin antagonists, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin (BKA1), 10 and 30μM,

and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (BKA3), 10 and 30μM both caused a dose-related histamine release from rat peritoneal mast cells following a 2 minute incubation, showing similar potencies (Tables 6.2 and 6.4). However, the bradykinin antagonist, [1-adamantaneacetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (BKA2) 10 and 30μM induced relatively less histamine release than the other kinins; bradykinin being approximately twice as potent than this compound. In addition, BKA2 was shown to antagonize the histamine releasing action of bradykinin (Table 6.3).

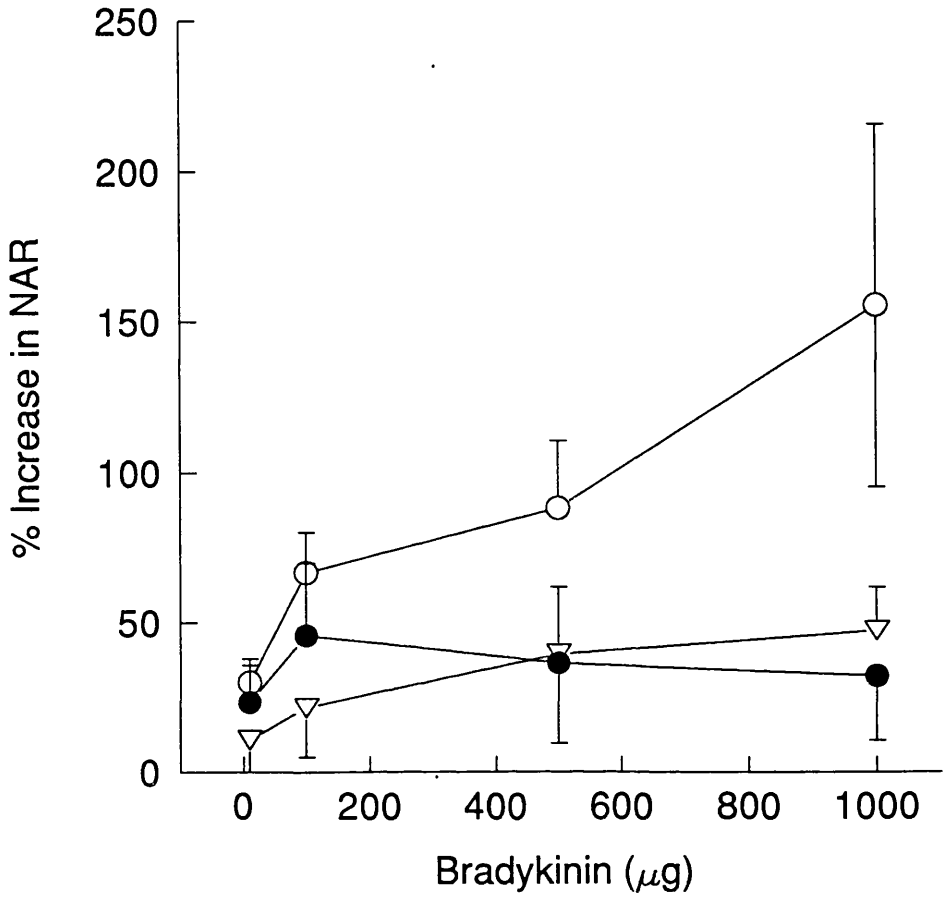


Figure 6.1

Effect of H_1 receptor antagonists on the dose-response curve for bradykinin and increased nasal airway resistance (NAR).

○ - no pretreatment (n=8); ∇ - subjects pretreated with terfenadine, 60mg, orally 3h before bradykinin challenge (n=6); ● - subjects pretreated with cetirizine, 10mg orally, 3h before bradykinin challenge (n=6). Vertical error bars represent s.e.m. Control (vehicle) values for NAR were: bradykinin alone, 0.320 ± 0.019 Pa.s.cm^{-3}; bradykinin with terfenadine, 0.372 ± 0.028 Pa.s.cm^{-3}; bradykinin with cetirizine, 0.392 ± 0.30 Pa.s.cm^{-3}.

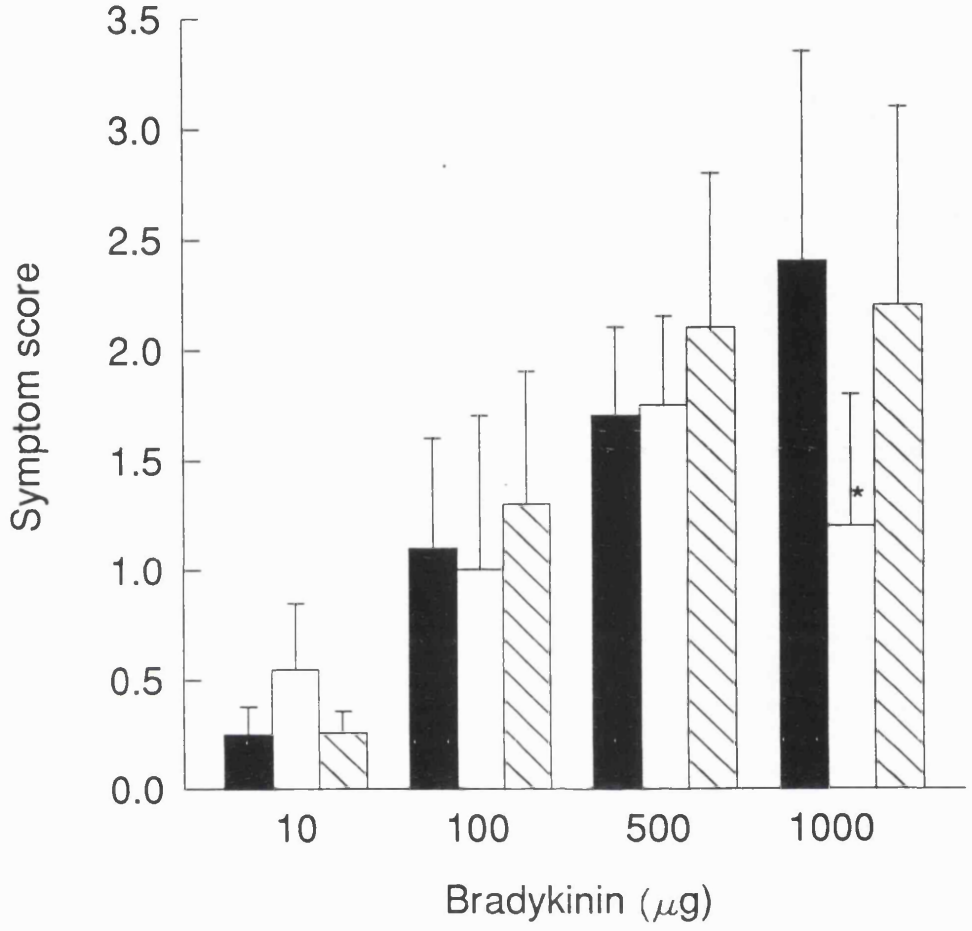


Figure 6.2

Dose-response relationship for bradykinin and total symptom scores in the presence and absence of H_1 receptor antagonists.

■- no pretreatment (n=8); □- subjects pretreated with cetirizine, 10mg orally 3h before bradykinin challenge (n=5); ▨- subjects pretreated with terfenadine, 60mg orally 3h before bradykinin challenge (n=6). *-significant difference ($p<0.05$).

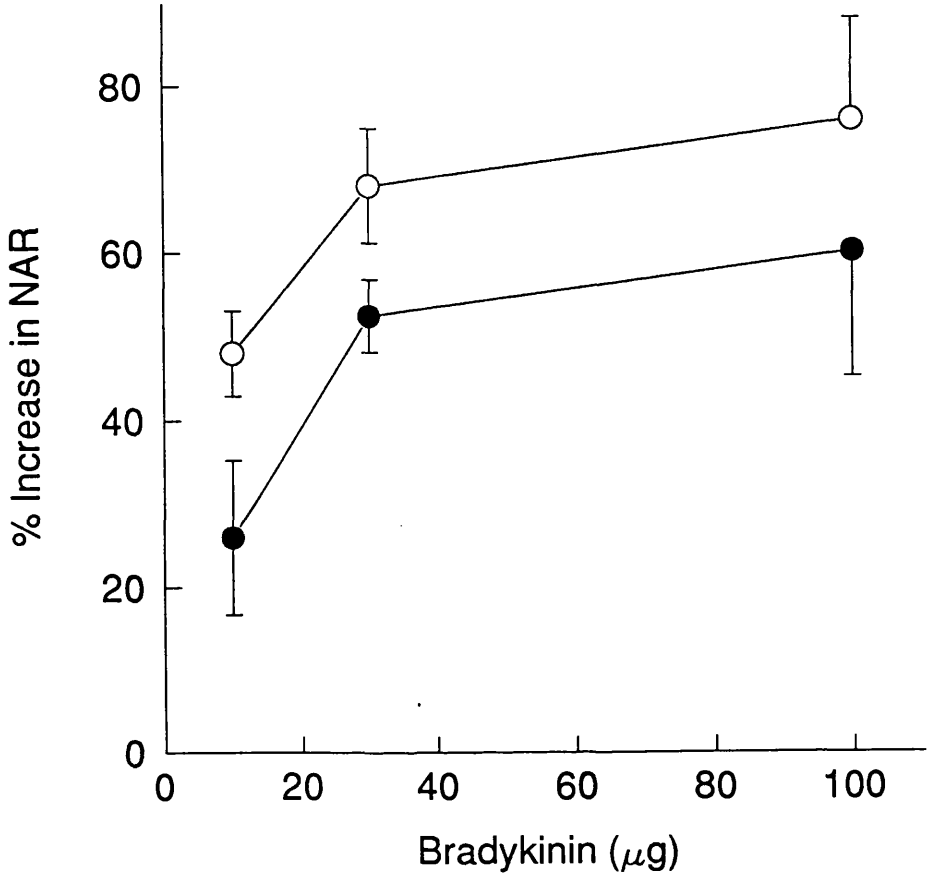


Figure 6.3

Dose-response curve for bradykinin and nasal airway resistance (NAR) at lower effective bradykinin concentrations in the presence of cetirizine, 20mg.

○ - no pretreatment (n=5); ● - subjects pretreated with cetirizine, 20mg orally, 3h before -bradykinin (n=5). Vertical error bars represent the s.e.m. Control (vehicle) values of NAR were: bradykinin alone, 0.324 ± 0.017 Pa.s.cm^{-3}; bradykinin with cetirizine, 0.399 ± 0.016 Pa.s.cm^{-3}.

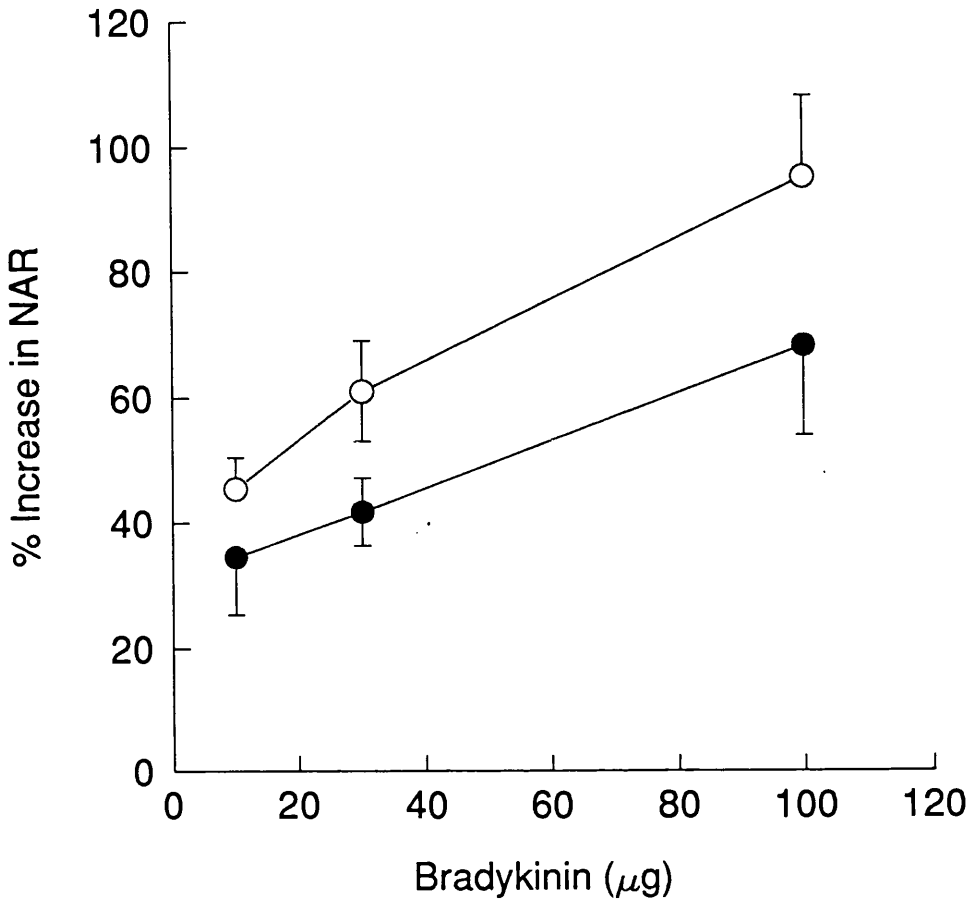


Figure 6.4

Dose-response curve for bradykinin and increase in NAR at the lower effective bradykinin concentrations.

○- no pretreatment , n=5; ●- pretreatment with cetirizine, 10mg, orally 3h before bradykinin challenge, n=6). Vertical error bars represent the s.e.m. Control (vehicle) values of NAR were: bradykinin alone, 0.298 ± 0.014 Pa.s.cm^{-3}; bradykinin with cetirizine, 0.360 ± 0.021 Pa.s.cm^{-3}.

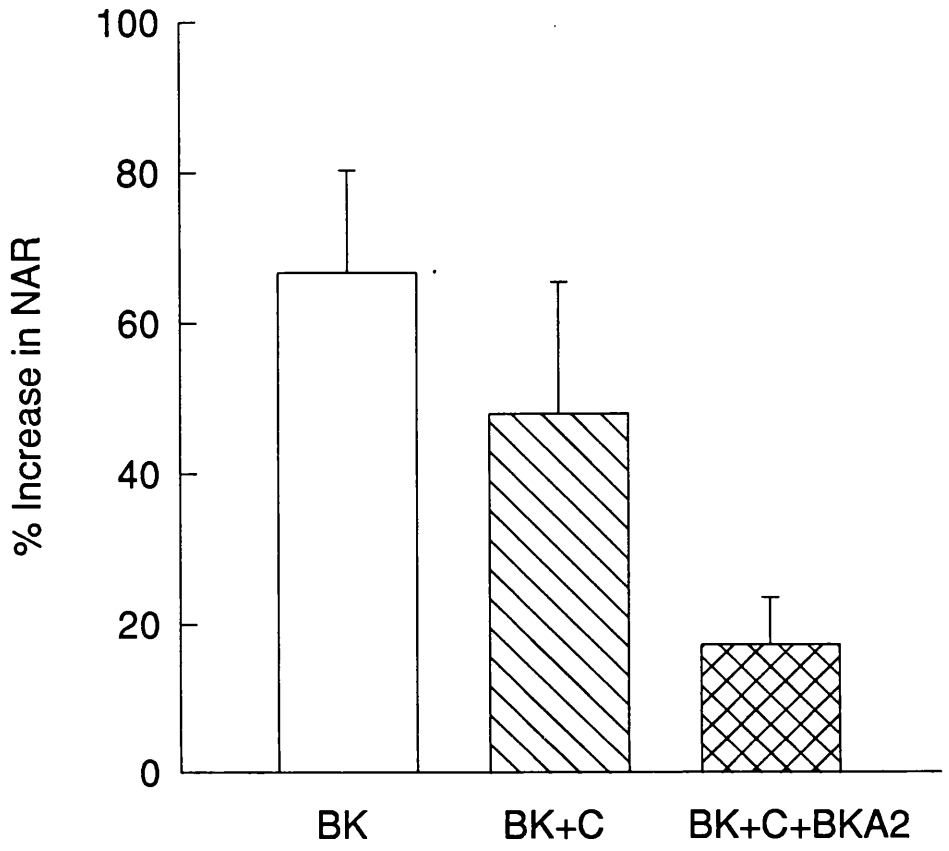


Figure 6.5

Effect of bradykinin, 100µg on NAR in the presence of cetirizine (C), 10mg and in the presence of [1-adamantane acetyl-D-Arg^{0},Hyp^{3},Thi^{5,8},D-Phe^{7}]-bradykinin (BKA2), 100µg plus cetirizine, 10mg.

□- bradykinin alone, 100µg; ▨- bradykinin, 100µg and cetirizine, 10mg; ▩-bradykinin, 100µg plus BKA2, 100µg and cetirizine., 10mg. Vertical error bars represent the s.e.m. Control (vehicle) values of NAR were: bradykinin alone, 0.326 ± 0.021 Pa.s.cm^{-3}; bradykinin with cetirizine, 0.289 ±0.015 Pa.s.cm^{-3}; bradykinin with cetirizine and BKA2, 0.361 ±0.022 Pa.s.cm^{-3}.

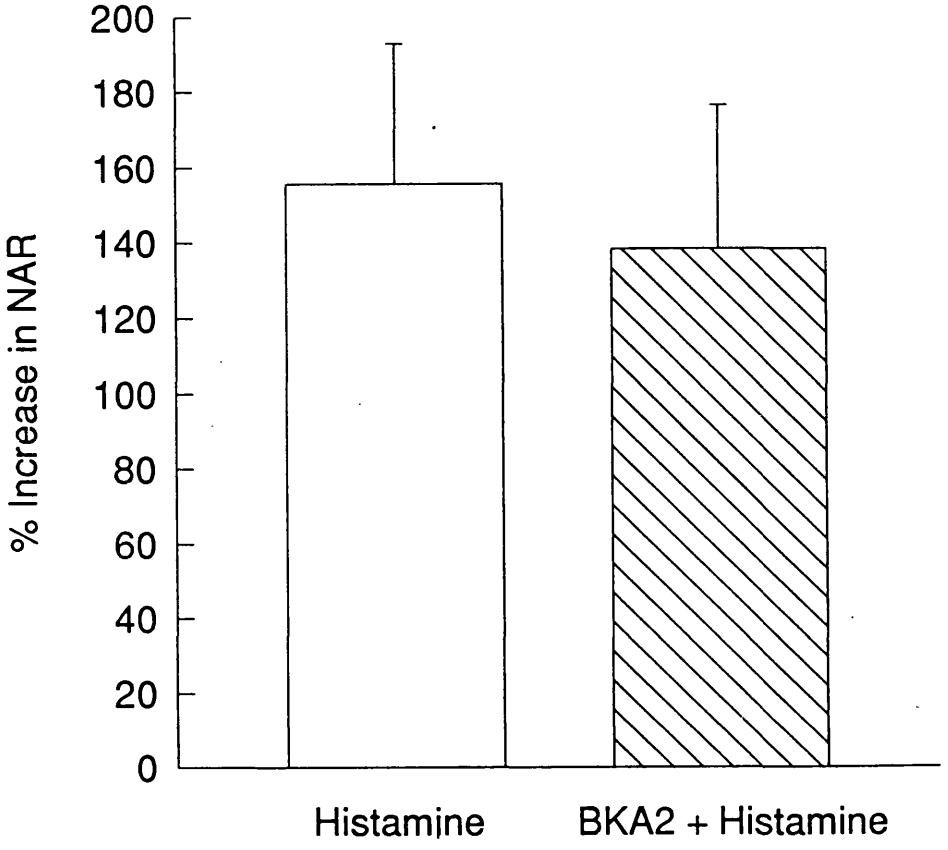


Figure 6.6

Effect of [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (BKA2) on histamine-induced increases in NAR.

□- histamine, 200μg; ▨- histamine, 200μg with pretreatment with BKA2, 100μg. The bars represent the means of 4 experiments. Vertical lines represent the s.e.m. The control (vehicle) values of NAR were 0.388 $\pm$ 0.04 Pa.s.cm^{-3} for histamine and 0.403 $\pm$ 0.09 Pa.s.cm^{-3} for histamine in the presence of the antagonist, BKA2.

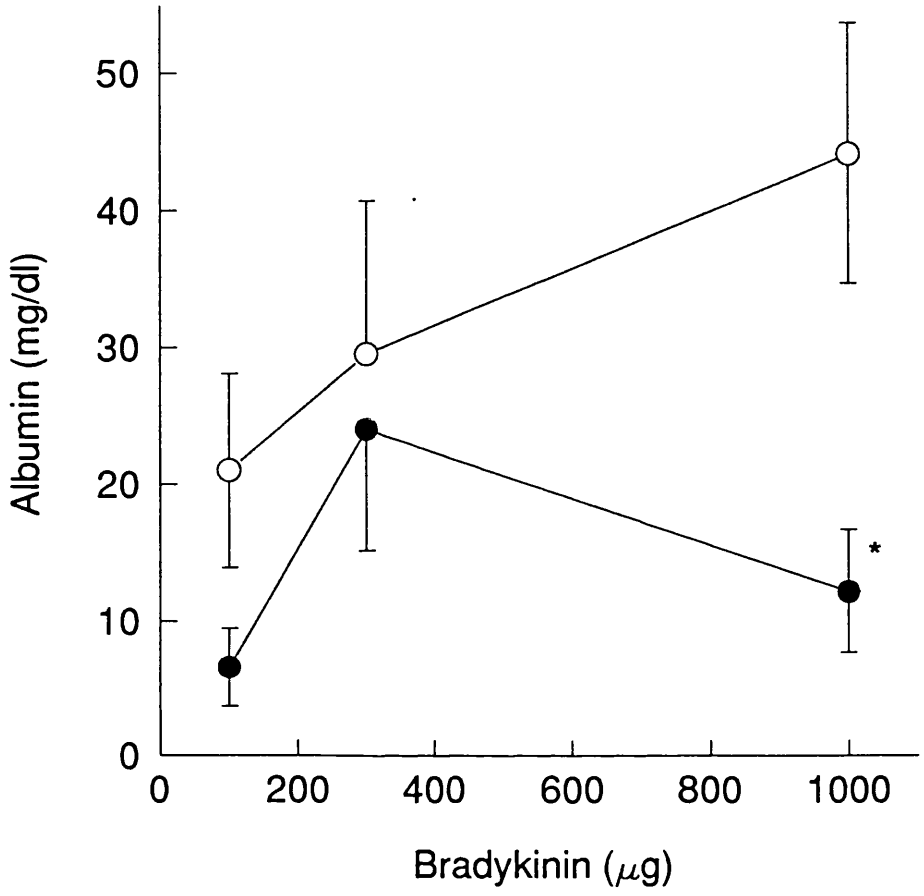


Figure 6.7

Dose-response curve for bradykinin and albumin concentration of nasal lavage.

○ - no pretreatment (n=10); ● - subjects pretreated with cetirizine, 10mg orally, 3h before bradykinin challenge (n=8). Vertical error bars represent s.e.m. *-significant difference ($p<0.05$).

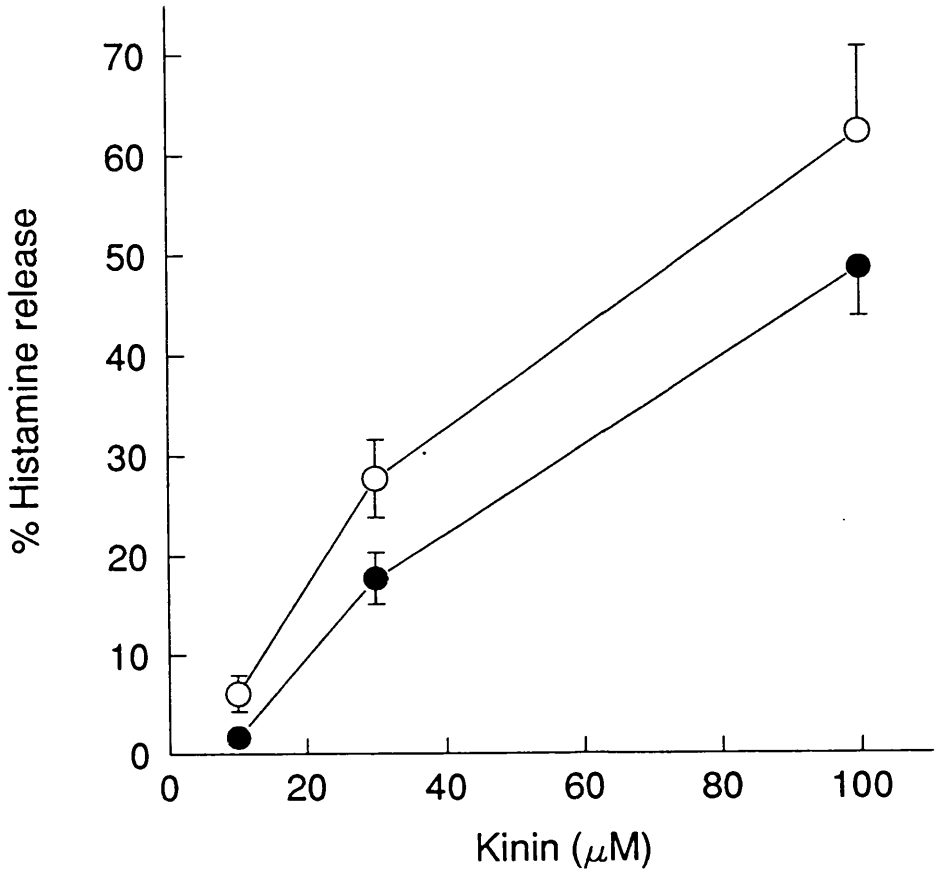


Figure 6.8

Dose-response curves for kinin-induced histamine release from rat peritoneal mast cells *in vitro*.

○ - bradykinin; ● - [Des-Arg^{10}]-kallidin. n=5 to 10. The data have been corrected by subtracting the histamine release from untreated cells which had a mean value of 6.1% for bradykinin and 4.8% for [Des-Arg^{10}]-kallidin.

Challenge agent	Saline (n=47)	Bradykinin, 300μg (n=15)	Bradykinin, 1000μg (n=32)
Histamine (nM) Mean $\pm$ S.E.M.	50.8 $\pm$ 8.0	58.9 $\pm$ 15.4	43.0 $\pm$ 9.4

Table 6.1

Effect of bradykinin on histamine concentration of nasal lavage

Bradykinin (μM)	Antagonist (μM) (BKA1)	Histamine release (% of total) Mean ± s.e.m.
10	-	2.9 ± 1.1
30	-	30.0 ± 6.7
-	10	10.0 ± 5.2
-	30	31.1 ± 4.7

Table 6.2

The effect of the bradykinin antagonist, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin (BKA1) on bradykinin-induced histamine release from rat peritoneal mast cells *in vitro*.

The cells were preincubated for 2 min. at 37^{0}C with the antagonist before challenge with bradykinin. The data are from 5 separate experiments each performed in duplicate. The data have been corrected by subtracting the histamine release from untreated cells which had a mean value of 5.0 ± 2.3% of total.

Bradykinin (μM)	Antagonist (μM) (BKA2)	Histamine release (% of total) Mean $\pm$ s.e.m.
10	-	10.0 $\pm$ 3.4
30	-	40.4 $\pm$ 2.6
-	10	2.4 $\pm$ 1.9
-	30	14.3 $\pm$ 6.1
10	10	2.4 $\pm$ 1.8
30	10	1.8 $\pm$ 1.2
10	30	12.1 $\pm$ 5.8
30	30	7.4 $\pm$ 5.8

Table 6.3

The effect of the bradykinin antagonist, [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (BKA2), on bradykinin-induced histamine release from rat peritoneal mast cells *in vitro*.

The cells were preincubated for 2 min. at 37^{0}C with the antagonist before challenge with bradykinin. The data are from 4 separate experiments each performed in duplicate. The data have been corrected by subtracting the histamine release from untreated cells which had a mean value of 7.0 $\pm$ 2.7% of total.

Bradykinin (μM)	Antagonist (μM) (BKA3)	Histamine release (% of total) Mean $\pm$ s.e.m.
10	-	7.5 $\pm$ 6.2
30	-	25.3 $\pm$ 6.0
-	10	7.4 $\pm$ 3.9
-	30	21.9 $\pm$ 6.2
10	10	19.0 $\pm$ 8.2
30	10	32.8 $\pm$ 8.0
10	30	24.1 $\pm$ 8.2
30	30	37.8 $\pm$ 7.5

Table 6.4

The effect of the bradykinin antagonist, [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (BKA3) on bradykinin-induced histamine release from rat peritoneal mast cells *in vitro*.

The cells were preincubated for 2 min. at 37^{0}C with the antagonist before challenge with bradykinin. The data are from 5 separate experiments each performed in duplicate. The data have been corrected by subtracting the histamine release from untreated cells which had a mean value of 9.1 $\pm$ 2.3% of total.

6.4 Discussion

The results described in this chapter show that intranasally administered bradykinin induces a dose-related increase in NAR and an increase in symptoms associated with nasal inflammation which can be partially inhibited by two chemically dissimilar H_1-receptor antagonists at normal therapeutic doses. This suggests that in the human nasal airway, bradykinin releases histamine from nasal mast cells or basophils. It is well established that histamine increases NAR by an action on H_1 receptors in the nasal airway (Britton *et al.*, 1978) and that this can be blocked by various H_1-receptor antagonists, including astemizole (Horak *et al.*, 1993) and cetirizine (Braunstein *et al.*, 1992; chapter 3). In this study, the fact that two chemically different histamine antagonists produced the same effect makes a non-specific effect of the drugs unlikely, particularly since cetirizine is a highly selective H_1-receptor antagonist (Snyder & Snowman, 1987). The ability of bradykinin to cause histamine release has been studied in various animal models of inflammation and by measuring histamine release in response to bradykinin, from mast cells *in vitro*. It has been shown that bradykinin-induced paw oedema in the mouse is completely inhibited by the H_1-antagonist, mepyramine (Wiseman *et al.*, 1994), although this is not so in the rat or guinea-pig (J. Wiseman, Glaxo Group Research Ltd., Ware-personal communication), and that antihistamines decrease bradykinin-evoked vascular permeability in rabbit skin (Marceau *et al.*, 1981b). In experiments where histamine release is measured from mast cells, bradykinin causes histamine release from rat peritoneal and mouse mast cells *in vitro* (Johnson & Erdos, 1973; Ishizaka *et al.*, 1985) but does not activate human basophils or human lung mast cells *in vitro* (Lawrence *et al.*, 1989). However, more recently it has been shown that the cutaneous vasodilatory effect induced by intradermal bradykinin in human skin, is in part, due to H_1-mediated histamine release (Li Kam Wa *et al.*, 1992). Therefore, it seems that bradykinin may have a differential effect in causing histamine release, depending on the species and tissue in question. Such heterogeneity of mast cell responses is well established (Barrett & Pearce, 1993).

A previous study which investigated the involvement of histamine in bradykinin-induced effects in the human nasal airway, described how terfenadine had no effect on bradykinin-

induced increases in NAR (Rajakulasingam *et al.*, 1992a). This contradicts the present findings in terms of NAR measurements. However, bradykinin was shown in the present studies to have no effect on histamine release into nasal lavage. This might suggest that bradykinin does not cause mast cell degranulation, consistent with the work of Rajakulasingam *et al.*, (1992a). The discrepancy between the effect of H_1 receptor blockade and measurements of NAR and the measurements of histamine release into nasal lavage described in this chapter, could occur for several reasons. It could be explained by a non-specific effect of the H_1-antagonists. Although this is highly unlikely because two different drugs have the same effect, it cannot be excluded, because it is impossible to be sure that cetirizine and terfenadine are only blocking histamine action. Alternatively, it could be that bradykinin does infact activate nasal mast cells in the nasal tissue and produce histamine-mediated effects, but that the histamine released is cleared into venous circulation and does not enter nasal cavity, so therefore is not detected in nasal washings. Furthermore, there may have been problems with the method used to measure histamine, either at the level of nasal lavage collection or the actual assay. It may be possible that a factor was present in the nasal lavage which interfers with the subsequent analysis of histamine. Several prewashes were undertaken to eliminate pre-existing histamine from the nasal cavity. Naclerio and co-workers have previously commented on the need for extensive prewashing of the nasal mucosa to reduce the baseline levels of lavage histamine to zero (Naclerio *et al.*, 1983a; 1983b). However, it is unclear as to whether the histamine detected after saline challenge was saline-induced or whether there is continuous release of histamine into the nasal cavity. Despite this, if bradykinin does release histamine into the nasal cavity, then one would expect this to still be detectable above whatever baseline is present. With regard to the assay, a reading was obtained from every sample measured and the duplicates were similar.

In an attempt to study bradykinin-induced histamine release further, experiments were performed using rat peritoneal mast cells. Ideally histamine release in response to incubation with bradykinin would be measured in human nasal mast cells, however, this was not possible since no human nasal tissue was available. It was decided to use rat peritoneal mast cells for these experiments since they have been shown to be a reasonable model to investigate peptide-induced histamine from human skin mast cells (Fewtrell *et*

al., 1982; Foreman *et al.*, 1982; Benyon *et al.*, 1987). It was found that bradykinin did indeed cause a dose-dependent histamine release from these cells, consistent with previous findings (Devillier *et al.*, 1988), as did two of the bradykinin antagonists employed.

Histamine release induced by bradykinin was first attributed to a receptor (Johnson & Erdos, 1973), which was thought to be of the B_2 type (Devillier *et al.*, 1985). However when it was discovered that two bradykinin analogues which are B_2 antagonists, also induced histamine release (Devillier *et al.*, 1988), the hypothesis was challenged. It was shown however, that the order of potency of bradykinin and bradykinin analogues in the induction of histamine release from mast cells is related to the number of positively charged amino acid resiues (Devillier *et al.*, 1985; 1988). This is illustrated by the results presented here. Bradykinin, with an arginine residue at both the N- and C-termini releases histamine, with similar potency to the B_2 antagonists, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin (BKA1) and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (BKA3), both of which have substituted arginine residues. However, acetylation of the N-terminal in the synthesis of [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (BKA2), results in a decrease of histamine-releasing activity. In the experiments described here, two of the antagonists studied, BKA1 and BKA3, have been shown to induce histamine release from rat peritoneal mast cells *in vitro*, but one of these (BKA3) is effective in blocking the bradykinin-induced increase in NAR. Therefore, if rat peritoneal mast cells *are* a model for kinin effects in nasal mast cells, then this implies that histamine release is not important with respect to the effect of the antagonists on bradykinin-induced increases in NAR. This point can really only be answered by performing similar histamine release experiments with human nasal mast cells.

The mechanism by which kinins release histamine from mast cells is actively being investigated. It is not kinin receptor-mediated since kinin antagonists do not inhibit histamine release, therefore the possibility of a kinin receptor-independent mechanism has been investigated. It appears that histamine release from mast cells is pertussis toxin senstive and consequently, the secretory activity of kinins on rat peritoneal mast cells has been attributed to the direct activation of G-proteins (Bueb *et al.*, 1990a). This proposed

mechanism is similar to that described for the wasp venom toxin, mastoparan and substance P (Mousli *et al.*, 1989). Bueb *et al.*, (1990b) have also shown that bradykinin and various analogues activate the GTPase activity of G-proteins purified from calf brain, with an order of potency corresponding to that observed on mast cell histamine release. Thus the direct activation of G proteins may be a receptor independent mechanism of mast cell activation by kinins.

Mast cells clearly belong to an heterogenous population, differing between species, and between organs within the same species. Kinin analogues are known to have differential histamine releasing effects between human cells, as illustrated by kinins causing histamine release from skin but not lung mast cells (Lawrence *et al.*, 1989). One study with human skin mast cells even reports individual heterogeneity of activation within a single population of cells from one donor (Cohan *et al.*, 1991). The use of rat peritoneal mast cells in the studies described above was the best model for mast cells in the human nasal airway available at the time and the results together with the *in vivo* results using H_1 antagonists provide some evidence for the effects of bradykinin in the nose being mediated, in part, by histamine. If this is so, then bradykinin may induce effects in the human nasal airway by both a direct receptor-mediated effect on the nasal vasculature and a receptor-independent effect on nasal mast cells.

SUMMARY

- The aim of this study was to investigate the contribution of histamine to the effects of bradykinin in the human nasal airway.

- Bradykinin-induced increases in NAR were attenuated by two chemically different H_1 receptor antagonists, cetirizine and terfenadine, at normal therapeutic doses.

- In the presence of ceterizine, the bradykinin antagonist, [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (BKA2), produced a further inhibition of the bradykinin-induced incease in NAR. The antagonist did not antagonize the action of histamine, 200μg on NAR.

- Bradykinin-induced increases in nasal vascular permeability, as quantified by albumin release in nasal lavage, were also attenuated by cetirizine, reaching statistical significance at bradykinin, 1000μg..

- Bradykinin 300μg and 1000μg had no effect on the histamine concentration of nasal lavage taken 10 minutes after challenge.

- Bradykinin and [Des-Arg^{10}]-kallidin, 10-100μM caused dose-related increases in histamine release from rat peritoneal mast cells *in vitro*.

- The bradykinin antagonists, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin (BKA1) and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (BKA3) both caused histamine release from rat peritoneal mast cells.

- The bradykinin antagonist, [1-adamantane acetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (BKA2), induced less histamine release from rat peritoneal mast cells relative to the other kinins, and also antagonized the histamine releasing action of bradykinin itself.

CHAPTER 7

The effect of platelet-activating factor on the responsiveness of the nasal airway in normal subjects

7.1 Introduction

Platelet-activating factor (PAF) is an extremely potent, endogenously produced phospholipid, which has been implicated in the pathogenesis of allergic airway disease. The biological activities of PAF, including its ability to increase vascular permeability, act as a potent chemoattractant for neutrophils and eosinophils, induce superoxide anion production and leukotriene synthesis (O'Flaherty *et al.*, 1983), suggest that it may be involved in the pathological events underlying asthma and rhinitis (see section 1.4.2). Furthermore, inhaled PAF has been shown to cause immediate bronchoconstriction in the lower airways (Chung *et al.*, 1986; Cuss *et al.*, 1986) as well as inducing a non-specific bronchial hyperresponsiveness in several species including guinea-pigs (Robertson *et al.*, 1988), dogs (Chung *et al.*, 1986), monkeys (Patterson *et al.*, 1984) and man (Cuss *et al.*, 1986). Such hyperresponsiveness is a characteristic feature of both asthma (Boushey *et al.*, 1980) and allergic rhinitis (Andersson *et al.*, 1987) and is one criterion for the diagnosis of these conditions.

Lyso-PAF and PAF are released in nasal secretions after local antigen stimulation in patients with hayfever (Miadonna *et al.*, 1989) thus indicating activation of the metabolic pathway leading to PAF generation. The authors found this was restricted to atopic patients, since no local mediator release was detected in the control group. Further evidence that PAF is a mediator of nasal hyperresponsiveness would depend, in part, on whether PAF applied to the normal nasal airway can mimic the hyperresponsiveness seen in allergic rhinitis. Such evidence exists for the lower airways (Cuss *et al.*, 1986). However, evidence implicating PAF as an important mediator in allergic rhinitis is not solely based on experimental models and studies with asthmatic patients.

The mechanism of PAF-induced hyperresponsiveness in the lower airway is not known. Many investigators have suggested that hyperrresponsiveness is a result of airway inflammation, but this is not entirely satisfactory, since asthmatic airways can remain hyperresponsive for years, in the absence of symptoms and mucosal inflammation (Townley *et al.*, 1975). However, one of the attractions of this hypothesis is that the inflammatory reaction has associated with it an influx of eosinophils which release cytotoxic proteins, such as eosinophil cationic protein and eosinophil peroxidase (Frigas & Gleich, 1986). These proteins are known to damage airway epithelium, thereby both increasing the accessibility for stimuli to the underlying structures in the nasal mucosa, and causing the epithelial desquamation characteristic of asthma airways (Holtzman *et al.*, 1983).

Eosinophils have been shown to generate oxygen metabolites (De Chatelet *et al.*, 1977), which together with the enzyme, eosinophil peroxidase and halide ions can form a potent cytotoxic system, which has been shown to have an injurious effect on human nasal epithelium *in vitro* (Ayars *et al.*, 1989). Furthermore, eosinophils from patients with allergic rhinitis appear to be "primed" and produce more oxygen metabolites, as measured by luminol chemiluminescence, in response to PAF than corresponding control groups (Shult *et al.*, 1986).

However, the situation regarding the mechanism of PAF-induced hyperresponsiveness is further questioned in the light of recent experiments showing that this occurs *in vitro*, predominantly in the absence of circulating inflammatory cells (Webber *et al.*, 1992). This hyperresponsiveness was suggested to be produced by the PAF receptor-mediated release of oxygen free radicals.

The following study was designed firstly, to determine whether PAF might be a potential mediator of hyperresponsiveness in the upper airway, by characterising the effects of intranasal PAF on the responsiveness of the human nasal airway in normal subjects; and secondly, to examine the possible contribution of oxygen free radicals in the PAF-induced events, by employing vitamin E as an oxygen free radical scavenger.

7.2 Experimental protocol

In each experiment, subjects were first given an intranasal aerosol of saline, then three measurements, each of four nasal cycles, were taken every 5 minutes by active posterior rhinomanometry (NAR) over a 15 minute period to provide the baseline (see section 2.2.2). To begin with, subjects were challenged with either four doses of histamine (50, 100, 200 and 400 μg) or bradykinin, 100 μg to each nostril. This dose of bradykinin had previously been shown to produce a significant decrease in nasal airway conductance (Proud *et al.*, 1988). After each dose, NAR was measured at 2, 5, 10, 15 and 20 min. Subjects were then treated with vehicle or PAF, 30μg or 60μg to each nostril and subsequent histamine or bradykinin challenges were performed at 2, 6, 24, 48 h and 7 days later. Subjects were allocated to PAF or vehicle treatment groups on a randomised basis. As part of a cross-over design, at least one week later, subjects were given vehicle or PAF; vehicle if they had previously had PAF and PAF if they previously had vehicle. Histamine or bradykinin challenges were performed again at 2, 6, 24, 48 h and 7 d after vehicle or PAF administration. In addition, subjects underwent a nasal lavage on each challenge occasion.

Another group of subjects were given lyso-PAF, 60μg to each nostril. A histamine challenge, 200μg was performed 6h later and NAR was measured as before over the 20 minutes post-challenge. Subjects also received identical histamine challenges with vehicle pretreatment. The challenges were separated by at least one week and allocation of subjects to a particular treatment was randomised.

In the study with vitamin E, 5 subjects received orally, vitamin E, 400 U daily for 14 days. A blood sample was taken from each subject immediately prior to starting the vitamin E treatment, and another sample was taken on day 14. At this time, subjects were treated with PAF, 60 μg to each nostril, as before, and histamine challenges (200μg) were performed at 2, 6 and 24 h after PAF pretreatment. Nasal lavages were also performed on these occasions, following NAR measurements.

7.3 Results

7.3.1 Characterisation of the nasal hyperresponsiveness induced by PAF

Inhalation of PAF alone caused no significant changes in NAR in any of the subjects. Similarly, no response was obtained following vehicle administration. All subjects sneezed and experienced nasal itching and nasal blockage after histamine challenge. However, histamine challenge before PAF (0h) and at 2, 6 and 24 h following PAF administration resulted in increases in NAR above baseline (saline control) of 79%, 162%, 215%, and 148% respectively, whereas responses to histamine with vehicle pretreatment were 79%, 89%, 74% and 91% respectively (n=5) (Figure 7.1). The maximum hyperresponsiveness induced by PAF was observed at 6h, when the percentage increase in NAR following histamine challenge was significantly larger than that obtained with vehicle administration ($P<0.05$, paired t-test between PAF and vehicle treatment) (Figure 7.1). Figure 7.2 shows the hyperresponsiveness to histamine at 6h over a dose range from 50 to 400μg, where the response appears to approach maximum.

Subjects challenged with bradykinin experienced nasal blockage (Figure 7.3) without other symptoms and this too was augmented following PAF treatment. Bradykinin-induced increases in NAR above baseline (saline) prior to PAF administration and at 2, 6 and 24 h after PAF were 27%, 32%, 92% and 28% respectively (n=5), as compared with increases of 27%, 30%, 38% and 31% when subjects received vehicle (Figure 7.3). There was no difference in the duration of the hyperresponsiveness induced by PAF between subjects challenged with histamine and those challenged with bradykinin (Figure 7.4).

Experiments designed to investigate the persistance of the hyperresponsiveness provided evidence of slight increased responsiveness to histamine remaining at 24h post-PAF, although this was not statistically significant. Nasal blockage following histamine challenge at 48 h and 7 days after PAF treatment was not significantly different from that when vehicle was administered (Student's t-test, $P>0.05$) (Figure 7.5).

The PAF-induced effect appears to be a dose-related phenomenon (Figure 7.6). A dose of 30 μg PAF shifted the dose-response curve to histamine to the left but the 60 μg dose produced a steeper dose-response curve which reached a maximum at a dose of histamine of 400 μg.

7.3.2. Effect of lyso-PAF on nasal responsiveness

Treatment of subjects with lyso-PAF, 60μg had no effect on nasal responsiveness to histamine, 200μg 6h after lyso-PAF. The response to histamine at this time was not significantly different from the response obtained following vehicle pretreatment. (n=5) (Figure 7.7).

7.3.3 Effect of PAF on eosinophil cationic protein concentration of nasal lavage fluid

The ECP concentrations in nasal lavage after PAF administration were significantly increased at 2h and 6h when compared with the ECP concentrations in the nasal lavage prior to PAF administration. The maximal increase in ECP concentration of the nasal lavage fluid, which occured at 6 h after PAF administration, was 8.3 μgl^{-1} compared with 2.9 μgl^{-1} before PAF treatment ($P<0.05$) (Figure 7.8).

7.3.4. Effect of vitamin E on PAF-induced nasal hyperresponsiveness

To study the effect of vitamin E on the PAF-induced hyperresponsiveness, subjects were pretreated with vitamin E 400 U for 14 d. In all subjects, serum levels increased significantly from a mean of 13.2 $\pm$ 1.02 mgl^{-1} to a mean of 18.2 $\pm$ 1.44 mgl^{-1} (table 7.1): a mean increase of 37.4 $\pm$ 5.2% ($P<0.01$). On day 14, the PAF-induced hyperresponiveness to histamine was inhibited (Figure 7.9), and there was also a corresponding reduction in ECP levels of nasal lavage fluid from 8.3 μgl^{-1} without vitamin E pretreatment, to 5.3 μgl^{-1} after vitamin E but this was only significant at the 6h time point (Figure 7.8).

	Vitamin E mgl^{-1} day 0	Vitamin E mgl^{-1} day 14
Subject 1	10.0	14.6
Subject 2	16.0	21.4
Subject 3	12.1	16.1
Subject 4	14.3	21.7
Subject 5	13.8	16.8
Mean $\pm$ s.e.m.	13.2 $\pm$ 1.02	18.2 $\pm$ 1.44

Table 7.1

Serum levels of vitamin E in 5 subjects before and after treatment with vitamin E, 400 U orally, daily for 14d.

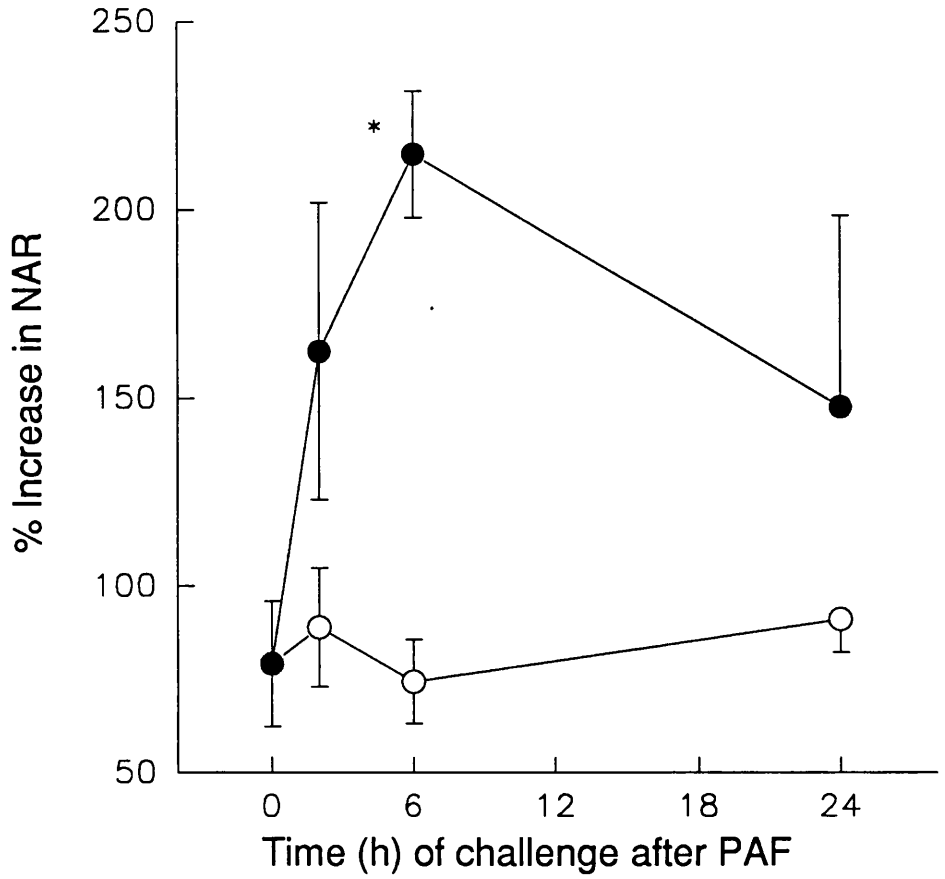


Figure 7.1

Time course for PAF-induced hyperresponsiveness to histamine.

Responses to histamine, 200μg expressed as maximal percentage increase in nasal airway resistance (NAR) above baseline in post-challenge period, before PAF (0h) and at 2, 6 and 24h following vehicle or treatment with PAF (60μg). (○) vehicle pretreatment; (●) PAF pretreatment. The baseline (saline) NAR was 0.317 $\pm$ 0.016 Pa.s.cm^{-3}. The data are the mean $\pm$ s.e.m from 5 separate experiments. * Significant difference ($P<0.05$) by paired t-test between PAF and vehicle pretreatment.

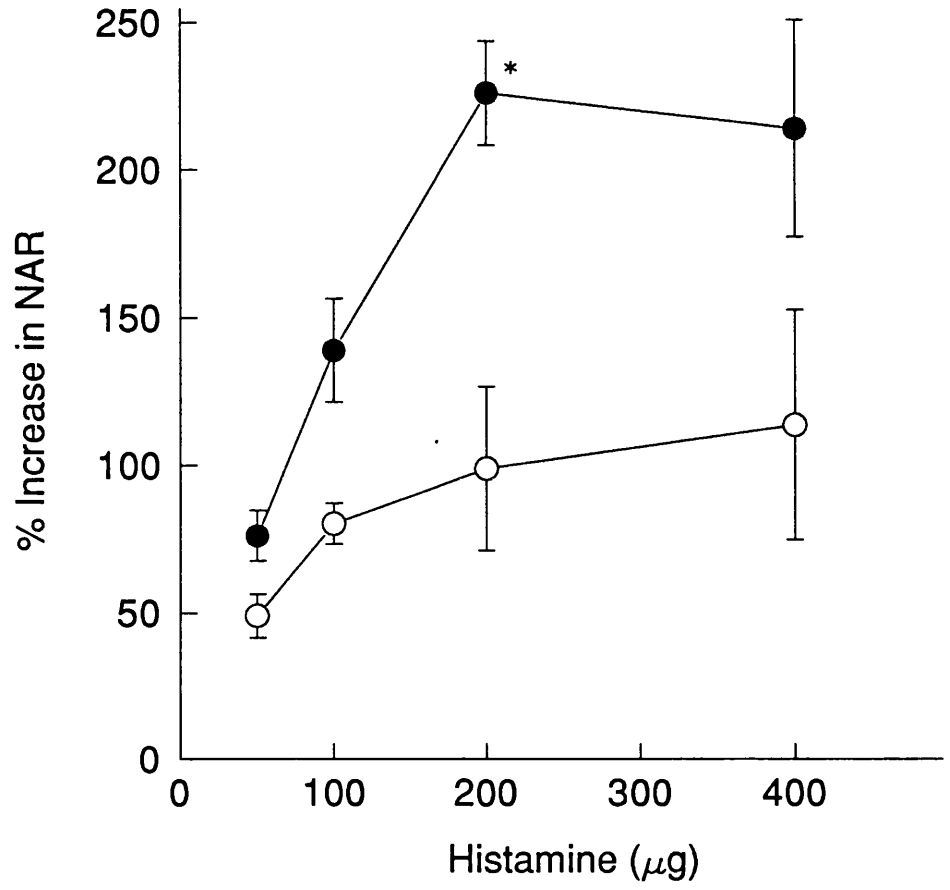


Figure 7.2

Dose-response curve for histamine and increased nasal airway resistance (NAR) 6h after PAF pretreatment.

Changes in NAR are expressed as a percentage of the baseline (saline) value which was 0.381 $\pm$ 0.021 Pa.s.cm^{-3}. (○) - vehicle pretreatment; (●) - PAF, 60 μg pretreatment. The data are the means $\pm$ s.e.m from 5 separate experiments. * Significant difference ($P < 0.05$) by paired t-test between PAF and vehicle administration.

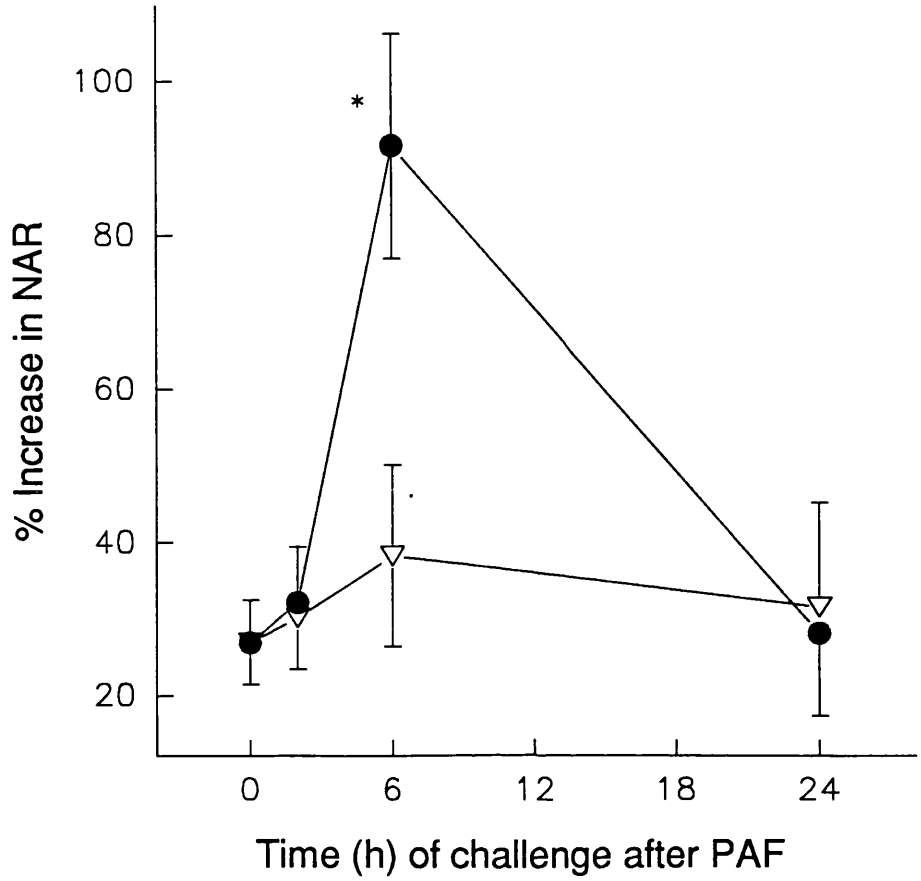


Figure 7.3

Time course for PAF-induced hyperresponsiveness to bradykinin.

Responses to bradykinin, 100 μg, expressed as maximal percentage increase in nasal airway resistance (NAR) above baseline (saline) in post-challenge period, before PAF (0h) and at 2, 6 and 24 h following vehicle or PAF pretreatment. (▽) - Vehicle pretreatment; (●) - treatment with PAF, 60μg. The baseline NAR was 0.343 $\pm$ 0.013 Pa.s.cm^{-3}. The data are the means $\pm$ s.e.m from 5 separate experiments. * Significant difference ($P < 0.05$) between vehicle and PAF administration.

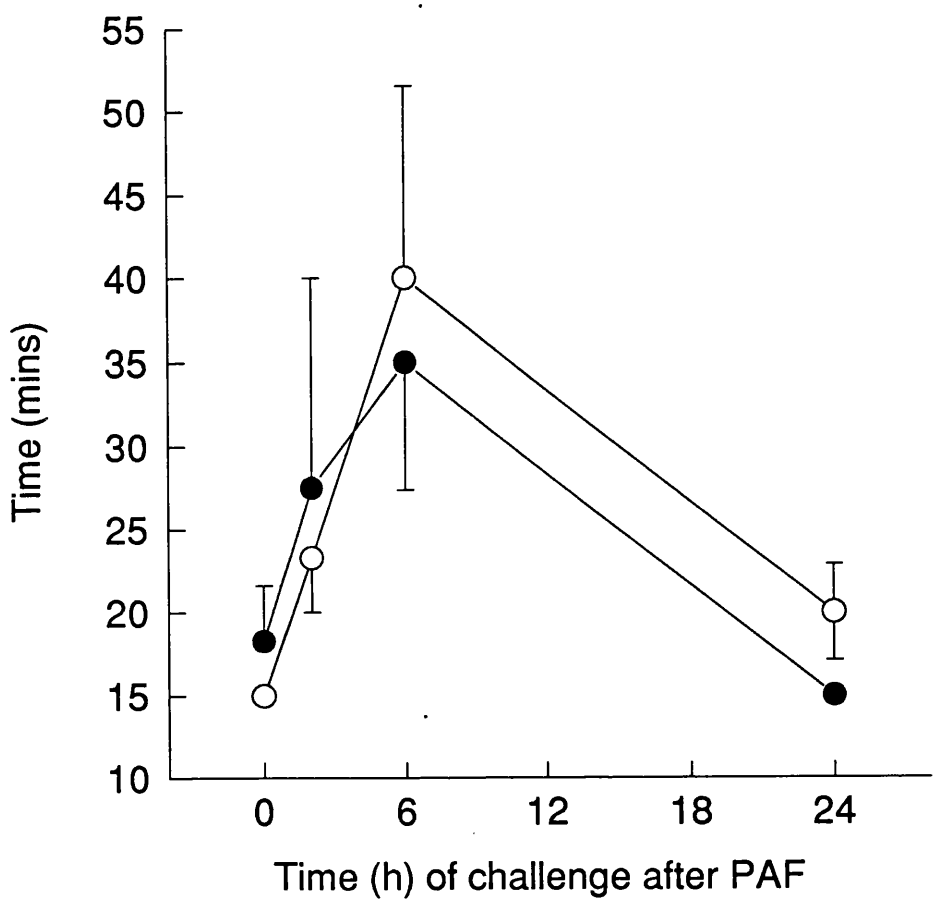


Figure 7.4

Time course for effect of PAF on duration of responses to histamine and bradykinin.

Total duration of response to histamine, 200 μg (●) and bradykinin, 100μg (○), before PAF (0h) and at 2, 6 and 24 h after administration of PAF, 60μg. The data are the means $\pm$ s.e.m from 5 separate experiments.

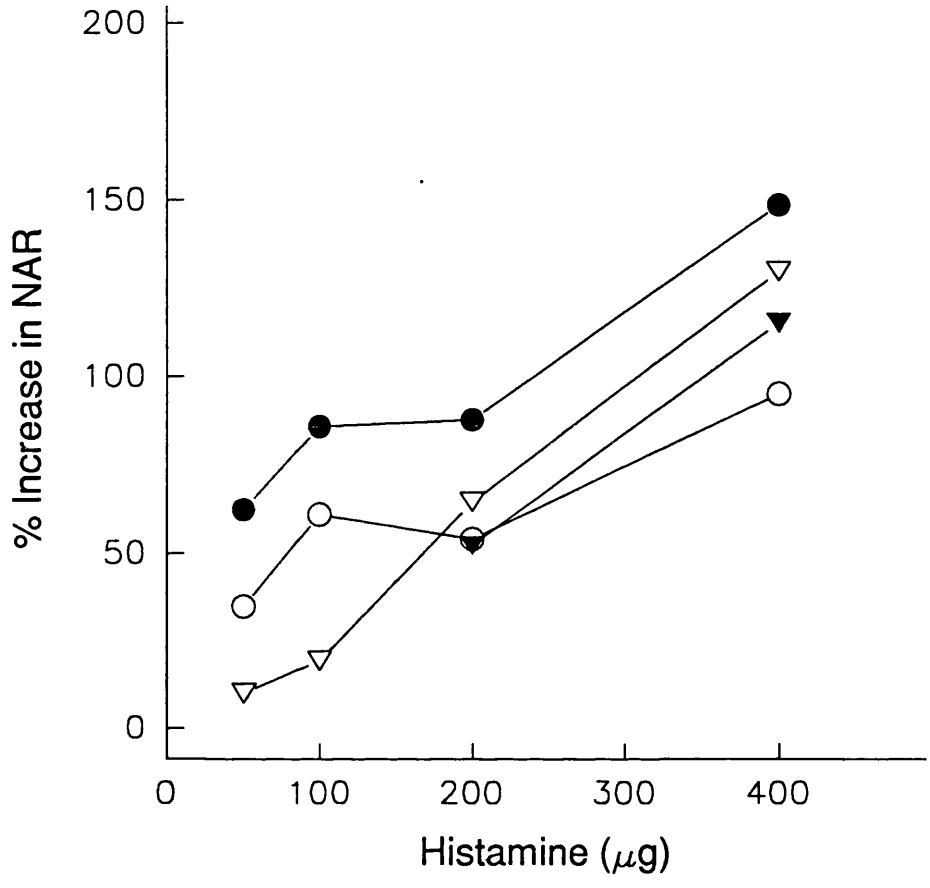


Figure 7.5

Dose-response curve for histamine and increased nasal airway resistance (NAR) before PAF (0h) and at 24h, 48h and 7 d after PAF, 30μg.

Changes in NAR are expressed as a percentage of the baseline (saline) value which was 0.339 $\pm$ 0.016 Pa.s.cm^{-3}. (○) - before PAF (0 h); (●) - 24 h after PAF; (▽) - 48 h after PAF; (▼) - 7 d after PAF. The data are the means from 6 separate experiments and bars representing the s.e.m. have been omitted for clarity.

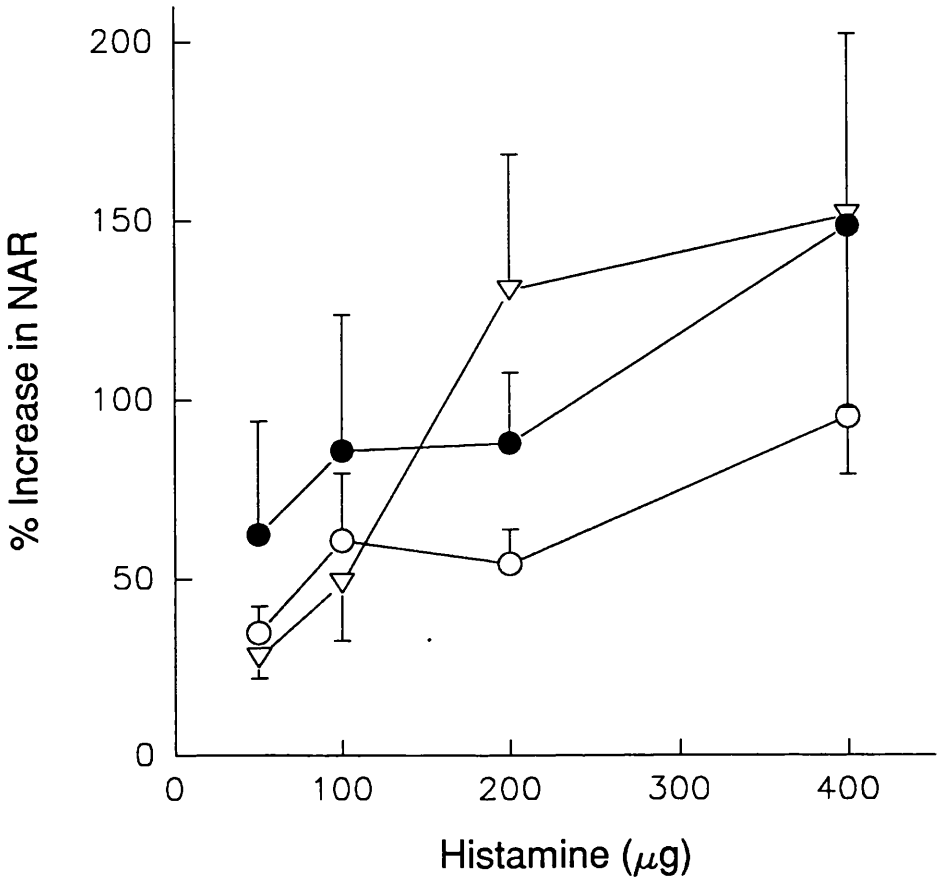


Figure 7.6

Dose-response curve for histamine and increased nasal airway resistance (NAR) at 24 h after PAF pretreatment.

Responses represented as maximal percentage change in NAR from the baseline (saline) of 0.301 $\pm$ 0.014 Pa.s.cm^{-3}. (○) - Control; (●) - PAF, 30 μg; (▽) - PAF, 60 μg. The data are the means $\pm$ s.e.m from 6 separate experiments.

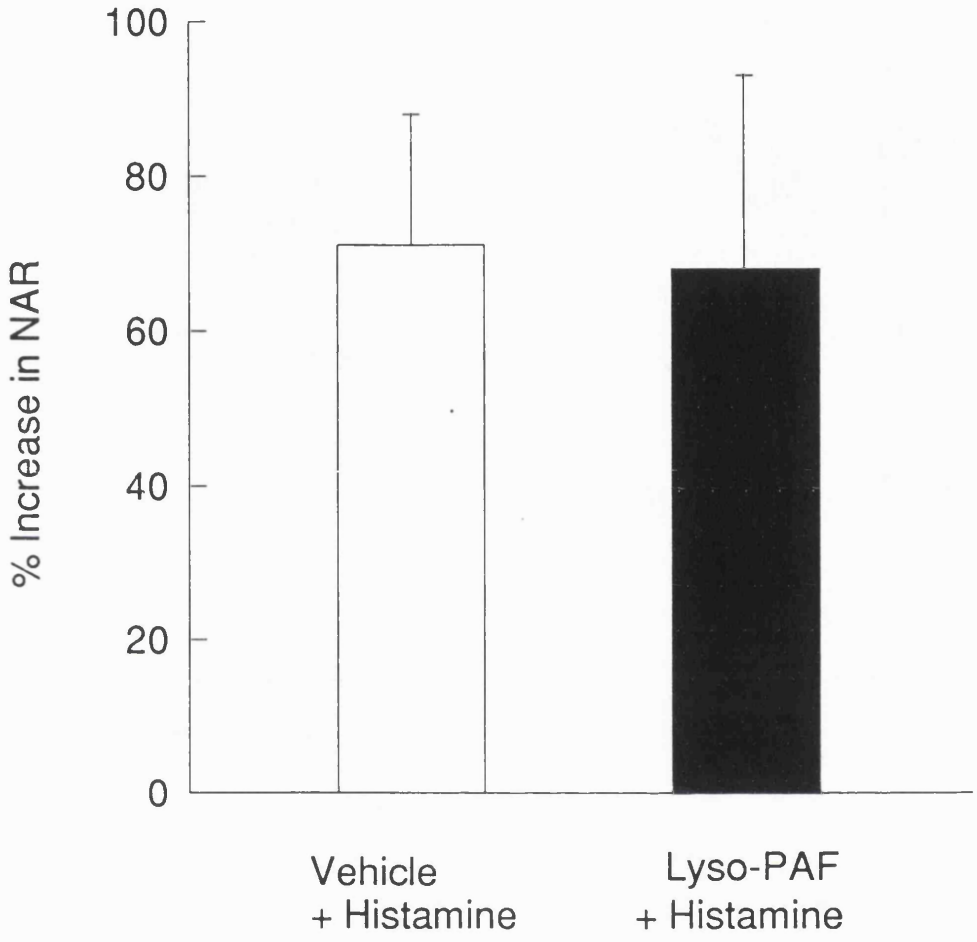


Figure 7.7

Effect of lyso-PAF on nasal responsiveness to histamine.

Changes in NAR to histamine challenge, 200μg 6h following administration of vehicle (□) or lyso-PAF, 60μg (■). All data expressed as percentage of baseline (saline) value which was 0.315 $\pm$ 0.015 Pa.s.cm^{-3}. The bars represent the mean from 5 seperate experiments. Vertical error bars represent s.e.m.

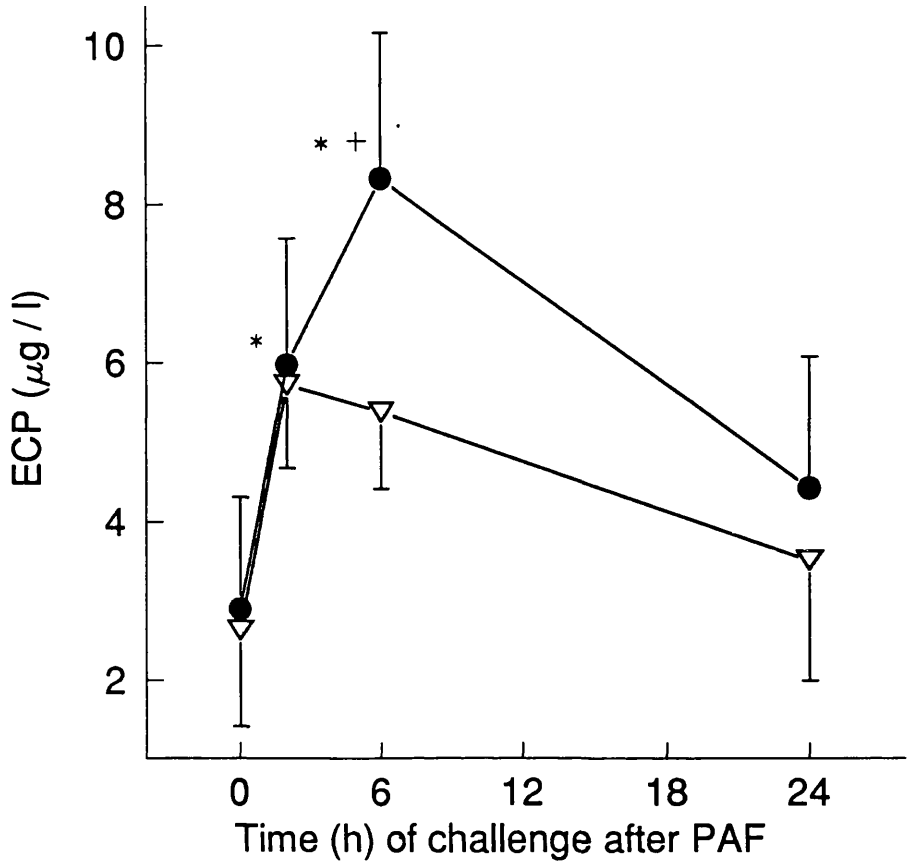


Figure 7.8

The effect of PAF administration on eosinophil cationic protein (ECP) concentration of nasal lavage fluid.

ECP concentration expressed as μgl^{-1} of nasal lavage taken before PAF (0h) and at 2, 6 and 24 h after administration. (●) - PAF, 60 μg; (▽) - PAF 60 μg and vitamin E treatment. The data are the means ± s.e.m from 3 separate experiments. * Significant change in ECP concentration compared with that before PAF administration (0h). + Significant difference in ECP concentration between the untreated subjects and those treated with vitamin E.

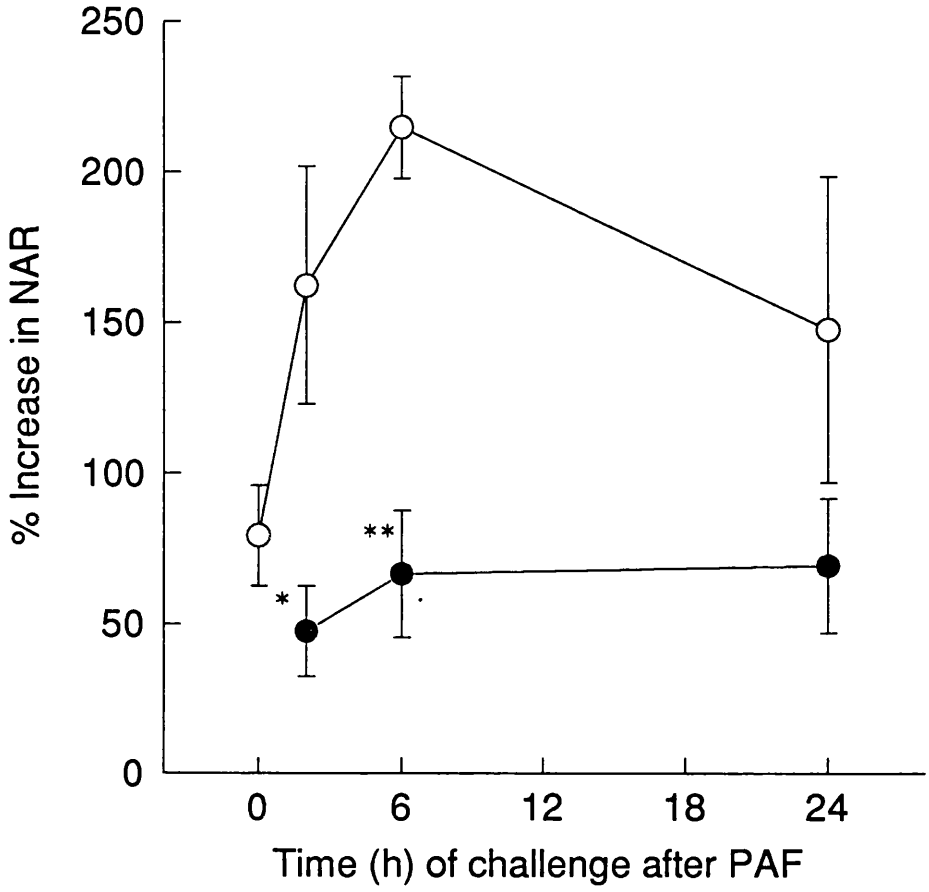


Figure 7.9

The effect of vitamin E pretreatment on the time course for PAF-induced hyperresponsiveness to histamine.

Responses to histamine, 200 μg, before PAF (0h) and at 2, 6 and 24 h after PAF, 60 μg administration, expressed as the maximal percentage increase in nasal airway resistance (NAR) above baseline (saline) during period after challenge with histamine. (○) - Control group; (●) - vitamin E pretreated group. The baseline NAR was 0.295 $\pm$ 0.022 Pa.s.cm^{-3}. The data are the means $\pm$ s.e.m from 5 separate experiments. Significant differences (* $P<0.05$ and ** $P<0.01$) between no treatment and vitamin E treatment.

7.4 Discussion

The experiments described above have demonstrated a non-specific increase in responsiveness induced by PAF in the human nasal airway. Control experiments have indicated that this effect is only induced by PAF, since both vehicle (saline) and lyso-PAF, both the precursor and metabolite of PAF (see section 1.5.2) had no effect on nasal responsiveness. This eliminates the possibility of the hyperresponsiveness being an effect secondary to the surfactant activity of the lipid. The PAF-induced hyperresponsiveness is non-specific in nature, as demonstrated by the increase in responses to both histamine and bradykinin following PAF administration. Hyperresponsiveness was present between 2 and 24h after PAF administration and was maximal at 6h. The dose of PAF was chosen from pilot experiments conducted prior to this study and also on the basis of studies by Andersson & Pipkorn (1988), who observed a small change in the nasal response to allergen challenge following a dose of 26μg PAF. The effects of the doses we have studied in the nasal airway are consistent with effects of PAF on the lower airway in humans (Cuss *et al.*, 1986), primates (Patterson *et al.*, 1984), dogs (Chung *et al.*, 1986) and guinea pigs (Chapman *et al.*, 1991).

The precise mechanism of PAF-induced airway hyperresponsiveness remains to be elucidated, although a variety of mechanisms have been suggested. The PAF-induced hyperresponsiveness has been shown to be dependent on platelet activation (Chapman *et al.*, 1985; Coyle *et al.*, 1990), and is thought to be linked to mediator release from inflammatory cells, particularly eosinophils (Frigas & Gleich, 1986; Sanjar *et al.*, 1990). So in addition to measuring nasal airway resistance, the possibility of eosinophil activation was also investigated.

The results show increases in ECP levels of nasal lavage fluid which have a similar time course to the PAF-induced hyperresponsiveness. This suggests that activation of eosinophils with subsequent release of ECP may contribute to the development of hyperresponsiveness. Indeed, the interest in examining eosinophil activation in these experiments initially arose from suggestions that airway hyperresponsiveness may, at least in part, be due to a change in the barrier function of the epithelium (Hogg, 1981), which

may be brought about by the release of cytotoxic constituents of eosinophils which in turn damage the airway epithelium.

However, examining the induction of hyperresponsiveness and ECP levels also addresses the controversy over whether hyperresponsiveness is independent of eosinophil activation, or whether there is a causal relationship between them. There is certainly abundant circumstantial evidence associating hyperresponsiveness with inflammatory cell recruitment and activation. This is largely derived from collecting inflammatory cells from airways, either via biopsy samples or as constituents of lavage fluid from patients with asthma. Despite this, more recent evidence from experimental models of the lower airways (Chapman *et al.*, 1991; Spina *et al.*, 1991; Herd *et al.*, 1992; Sasaki *et al.*, 1993) indicates that hyperresponsiveness and inflammation/eosinophilia may not be sequential events. These studies show pulmonary eosinophilia without attendant hyperresponsiveness and *vice versa*. Clinical studies with asthmatic patients provide further evidence for this (Lundgren *et al.*, 1988; Gibson *et al.*, 1989). In the human nasal airway, ECP levels in nasal secretions have been linked with allergen-induced nasal hyperresponsiveness (Linder *et al.*, 1987). However, others (Andersson *et al.*, 1989) found a wide variety of ECP levels with no clearcut relationship with the induction of hyperresponsiveness. Moreover, Klementsson *et al.* (1991) found that eosinophil counts and ECP levels did increase after the nasal allergic reaction, but this occured *after* hyperresponiveness was observed. It seems, therfore, that it is unlikely that eosinophil recruitment and activation is solely responsible for nasal hyperresponsiveness, although it might play a role at some stage in the chain of events.

Other hypotheses have been investigated, including the possibility of hyperresponsiveness being due to a change in the density or function of specific receptors in the airway, such as histamine or muscarinic receptors (Robertson *et al.*, 1988). However, this was found not to be the case, although receptor densities have not been examined in the nose. Therefore, the recent evidence that PAF-induced hyperresponsiveness occurs *in vitro*, largely in the absence of inflammatory cells, is intriguing. This is not affected by indomethacin, FPL55712 or mepyramine and cimetidine, but is prevented by catalase, superoxide dismutase (SOD) and WEB2086 (Webber *et al.*, 1992). It has been suggested

that a receptor-mediated release of oxygen free radicals may be responsible for this effect, since SOD destroys O_2^-.

Oxygen free radicals have been implicated in other pathological conditions, as demonstrated by the protective effect of SOD (Rubanyi & Vanhoutte, 1986). In the airway, several reports suggest that oxygen free radicals may be linked to the development of non-specific airway hyperresponsiveness: ozone inhalation can induce hyperresponsiveness in animals (Murlas & Roum, 1985) and man (Seltzer *et al.*, 1986); inhalation of xanthine and xanthine oxidase (an oxygen free radical generating system) has been reported to induce airway hyperresponsiveness in anaesthetised cats (Katsumata *et al.*, 1990); hydrogen peroxide can induce potentiation of contractile responses in isolated rat airway which can be inhibited by catalase (Szarek & Schmidt 1990), and xanthine oxidase activation is associated with respiratory viral infection (Akaike *et al.*, 1990). In addition, ascorbic acid reduces methacholine-induced bronchoconstriction in hyperresponsive asthmatic subjects (Mohsenin *et al.*, 1983).

In this study, the role of free radicals in PAF-induced hyperresponsiveness in the nasal airway was examined using vitamin E as a free radical scavenger. Treatment with vitamin E inhibits the PAF-induced increase in responsiveness to histamine and also the increase in ECP in the nasal lavage. The dose of vitamin E used has previously been shown to produce a maximum increase in serum levels (Szczekilk, 1987). Vitamin E is a well-documented, effective antioxidant; protecting unsaturated lipids against peroxidation and free radical formation. There is evidence that vitamin E has a similar protective effect in tissues (McCay *et al.*, 1972). It acts by reacting with and stabilizing free radicals to prevent the chain of events by which they lead to cell damage (Fragata & Bellemare, 1980). Assuming that the levels of vitamin E produced in the subjects are blocking only free radical formation, then these data suggest that PAF-induced free radical formation is responsible for both hyperresponsiveness and eosinophil activation.

Therefore, these results support the hypothesis that hyperresponsiveness may be an event which is *parallel* to eosinophil activation. If this true, then it does not exclude the idea that the integrity of the epithelium is important in determining the extent of

hyperresponsiveness. Epithelial shedding in asthmatic airways or epithelial damage in the nose could easily be responsible for hyperresponiveness. This hypothesis is somewhat attractive if one considers that it could be free radical species that initiate the epithelial damage, and because hyperresponsiveness is only apparent in tissue where the epithelium was originally intact (Flavahan *et al.*, 1988). Therefore, one hypothesis could be that PAF induces oxygen free radical formation via a receptor mediated mechanism and that these species cause detrimental changes in the epithelial layer. The source of the free radicals may be the epithelium itself (Webber *et al.*, 1992). *In vivo*, production of oxygen metabolites in this way, may also activate nearby cells, causing further release of free radicals, forming a self-perpetuating cascade.

The results presented here show that hyperresponsiveness is virtually abolished by the antioxidant, vitamin E. This certainly supports several reports in the literature implicating free radical involvment in hyperresponsiveness. However, *in vivo*, considering the possible interactions between the airway, circulating cells and mediators, it is quite likely that hyperresponsiveness is due to several factors. In the human nasal airway, there is no further information on this. Similarly, there is a lack of information about the mechanism by which oxygen free radicals may cause hyperresponsiveness. However, it is interesting to consider two studies in particular which could be relevant to the nasal airway. Firstly, oxygen metabolites may inhibit neutral endopeptidase in the epithelium, which normally degrades tachykinins and bradykinin, thus resulting in exaggerated responses to these endogenous peptides (Yeadon *et al.*, 1990). Secondly, oxygen radicals, from lipid peroxidation, may provoke the release of arachidonic acid from membrane phospholipids and therefore lead to the release of prostaglandins and leukotrienes (Hemler *et al.*, 1979), both of which have the potential to exert quite potent effects in the nose (see section 1.5.4).

Finally, pharmacological blockade of nasal hyperresponsiveness clinically, can be achieved with topical glucocorticosteroids (Pipkorn *et al.*, 1987a; 1987b). In this context it is of interest that glucocorticosteroids can block the generation of PAF *in vitro* from isolated mouse mast cells (Benhamon *et al.*, 1986) and *in vivo*, after allergen challenge in guinea-pigs (Chignard *et al.*, 1986).

SUMMARY

- The effects of the topical application of an aerosol of platelet-activating factor (PAF) on responsiveness of the human nasal airway were examined in normal subjects. Nasal airway resistance was measured in response to histamine and bradykinin at 2, 6, 24, 48h and 7 d after PAF pretreatment. Eosinophil cationic protein (ECP) in nasal secretions was also measured.

- Intranasal aerosol administration of PAF, 30 or 60 μg per nostril to normal human subjects induced an increased responsiveness to aerosol administration of histamine, 50 to 400μg and bradykinin, 100 μg per nostril at 2, 6 and 24h following PAF treatment. However, the effect was not apparent at 48h or 7 days after PAF pretreatment.

- There was no difference in the time course of the PAF-induced hyperresponsiveness to histamine or bradykinin.

- PAF-induced nasal hyperresponsiveness at 2, 6 and 24 h was associated with increases in the ECP concentration of the nasal lavage fluid.

- Vitamin E pretreatment of subjects resulted in the attenuation of the PAF-induced hyperresponsiveness to histamine, and a similar decrease in ECP levels of the nasal lavage fluid.

- The results suggest that in the human nasal airway, PAF induces a non-specific hyperresponsiveness which is accompanied by eosinophil activation in the nasal cavity. Free radical production induced by PAF may contribute to the hyperresponsiveness and to the activation of eosinophils.

CHAPTER 8

A guinea-pig model for the quantitative determination of vascular permeability in the nasal mucosa.

8.1 Introduction

A reliable experimental model of rhinitis using laboratory animals would be a useful tool for the pharmacological evaluation of putative mediators or drugs which are thought to have either a role in the pathophysiology of rhinitis or a particular therapeutic application. To date, only a few models have been reported (Misawa, 1988; Misawa & Iwamura, 1990; Mizuno *et al.*, 1990), which is reflected by the lack of fundamental experimental studies on allergic rhinitis. This chapter concerns an attempt to develop a guinea-pig model for quantifying vascular permeability of the nasal mucosa in response to kinin challenge, with a view to using this model for further investigation of other inflammatory mediators.

Increases in vascular permeability refer to an alteration in the blood-tissue barrier provided by the wall of certain vessels of the microcirculation. Normally, this barrier is freely permeable to water, electrolytes and small molecules, but only slightly permeable to proteins. However, an inflammatory reaction, such as that which may occur in allergic rhinitis, often features enhanced vascular permeability and this corresponds to an accelerated rate of passage of plasma proteins into the extravascular environment. It has been shown that synthetic diazo dyes, such as Evan's blue, trypan blue or pontamine sky blue, given intravenously, bind spontaneously and quantitatively to plasma proteins, in particular to albumin (Rawson, 1943; LeVeen & Fishman, 1947). Therefore, appearance of such a dye in a sample of lavage or perfusate, indicates exudation of plasma proteins and enhanced vascular permeability.

The aim of the following experiments was to develop a model where the nasal cavity

could be perfused in a manner which facilitated the collection of nasal lavage samples. In the present study, it was intended that the Evan's blue concentration of the samples would be measured in order to quantify vascular permeability. The model developed was required for future use in the analysis of vascular permeability induced by a variety of substances, such as histamine, eosinophil proteins, leukotrienes, and kinins.

8.2 Experimental protocol

The perfusion circuit was filled with saline and the nasal passages were perfused for 25 minutes, to allow for equilibration. Five collections and replacements of perfusion fluid were made during this time, to achieve a stable basal leakage of Evan's blue dye. Saline (control) followed by [Des-Arg^9]-bradykinin (1μM final concentration) or bradykinin, (10nM, 0.1μM or 1μM, final concentrations) was administered in a volume of 25μl to the perfusion circuit. Every 5 minutes for 30 minutes, the perfusion fluid was collected and the circuit refilled.

The data was analysed statistically, by one-way analysis of variance (ANOVA) and by paired t-tests where appropriate.

8.3 Results

Administration of saline to the perfusion circuit was associated with small fluctuations in Evan's blue leakage during the 30 minute time-course (Figure 8.1). However, these did not reach statistical significance ($n=5$; ANOVA $p>0.05$).

Figure 8.1 also shows that the B_1 agonist, [Des-Arg^9]-bradykinin, 1μM had no significant effect on Evan's blue leakage ($n=5$; ANOVA $p>0.05$).

Bradykinin, 10nM, 0.1μM and 1μM, caused increases in vascular permeability as quantified by the increased Evan's blue measured in the perfusate. Figure 8.2 shows that bradykinin, 10nM induced an increase in Evan's blue leakage which was maximal at 20 minutes ($n=8$; paired t-test, $p=0.04$). The leakage was only significantly different from the control (saline challenge) at this time point. Bradykinin, 0.1μM and 1μM caused an increase in Evan's blue leakage which was maximal at 10 minutes for both doses, but did not reach statistical significance ($n=4$; $p>0.05$; $n=5$; $p>0.05$ respectively). The Evan's blue leakage was less in magnitude than that induced by bradykinin, 10nM.

Figure 8.3 shows the dose-response curve for bradykinin and Evan's blue leakage. There appears to be a negative relationship between dose and response, but statistically, there was no significant difference between the effect of the doses on Evan's blue leakage (ANOVA, $p>0.05$).

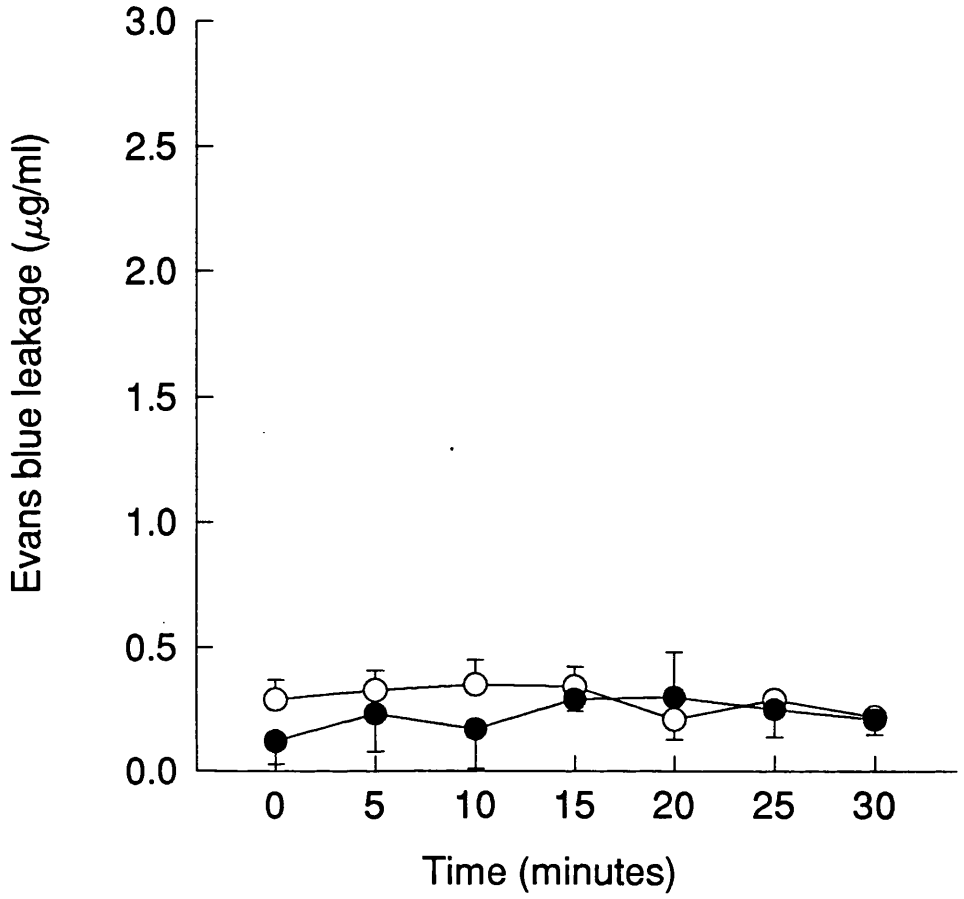


Figure 8.1

The effect of [Des-Arg^9]-bradykinin on Evan's blue leakage into nasal perfusate in the guinea-pig.

Evan's blue dye leakage expressed as μg/ml of perfusate following saline (●) and [Des-Arg^9]-bradykinin, 1μM (○) administration to the circuit. Samples of perfusate were taken every 5 minutes for 30 minutes. The data are the means from 5 experiments for each and the vertical bars represent the s.e. m.

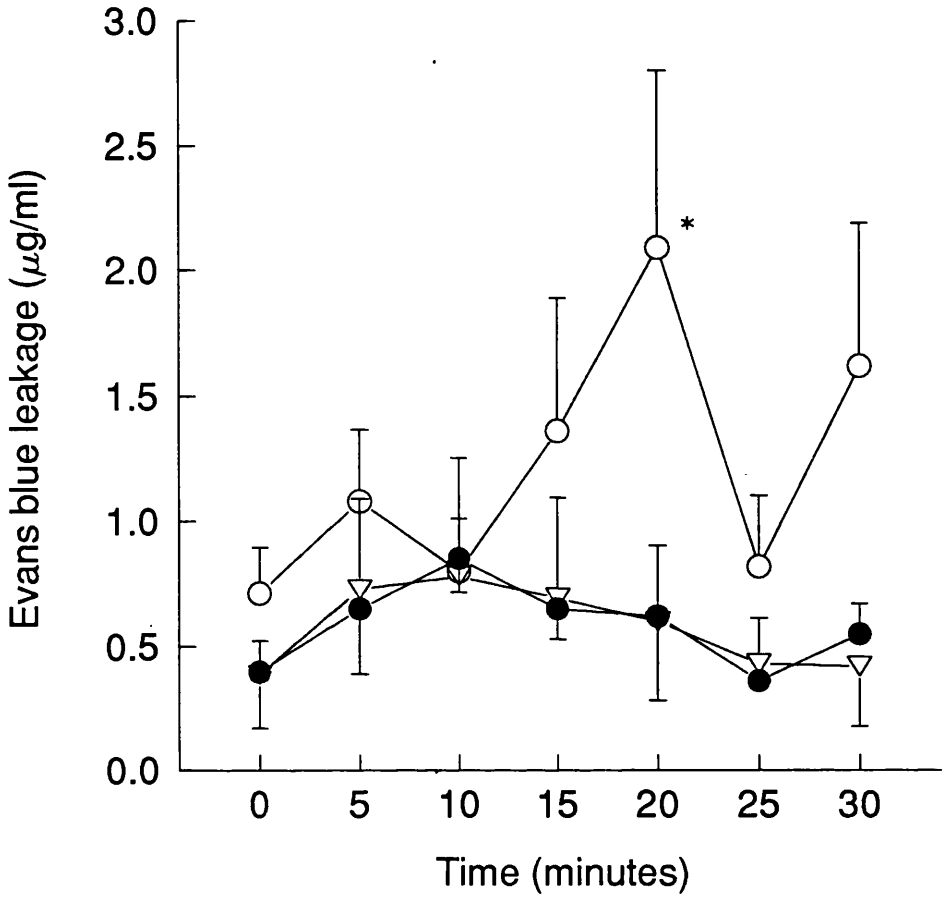


Figure 8.2

Time course for bradykinin-induced Evan's blue leakage into nasal perfusate in the guinea-pig.

The effect of bradykinin during the 30 minute post-challenge period on Evan's blue leakage into the perfusion fluid. Bradykinin, 10nM (○); 0.1μM (●) and 1μM (▽). The data are the means from 4 to 8 experiments and the vertical error bars represent the s.e.m. * - significantly different from control (saline).

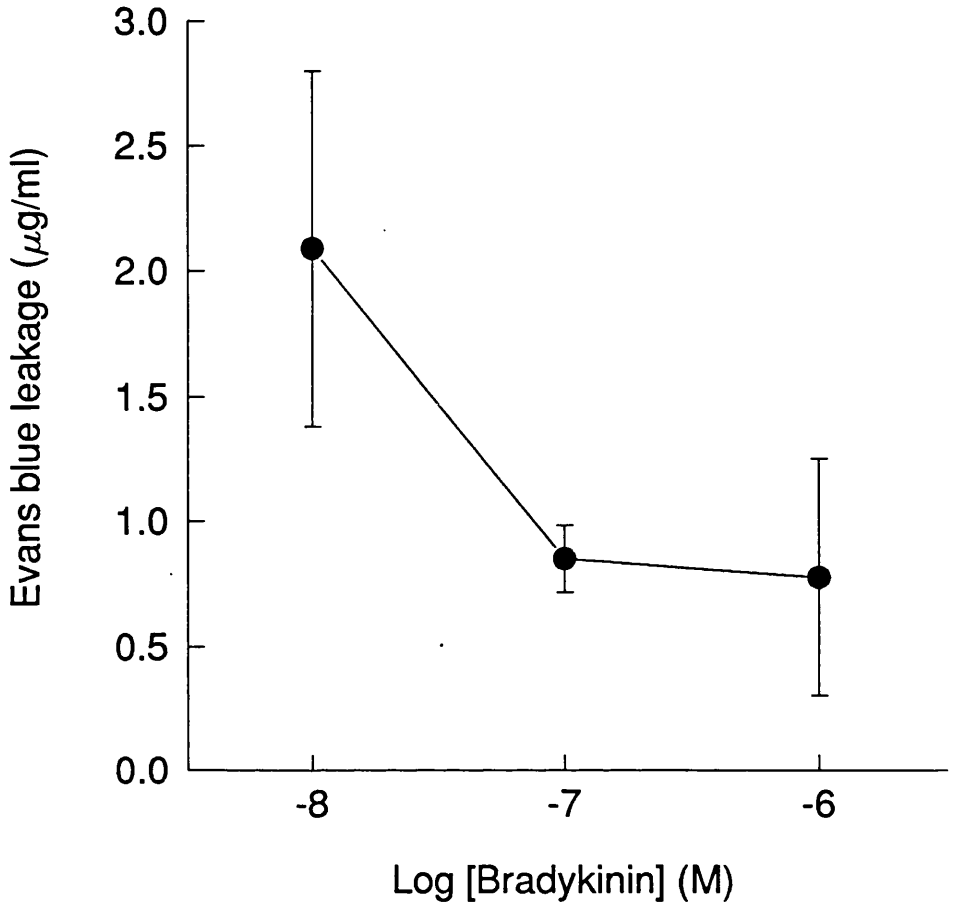


Figure 8.3

Dose-response curve for bradykinin and Evan's blue leakage into nasal perfusate in the guinea-pig.

Evan's blue leakage expressed as μg/ml of perfusate. The data are the means from 4 to 8 experiments and the vertical error bars represent the s.e.m.

8.4 Discussion

Changes in vascular permeability occuring in the nasal mucosa is one of the features of the response to nasal bradykinin challenge (Proud *et al.*, 1988; chapter 4) and also occurs in experimentally-induced seasonal allergic rhinitis (Baumgarten *et al.*, 1985). An experimental model where vascular permeability could be measured would, therefore, be useful for further studies on kinin involvement in allergic rhinitis and studies on the role of other putative mediators of allergic rhinitis.

In the present study, a guinea-pig model was developed in which the nasal airways were perfused in a manner where perfusate could be collected at intervals for analysis. Evan's blue dye leakage was used as a marker for plasma exudation and therefore vascular permeability (Evans *et al.*, 1987). The technique depends upon binding *in vivo* of the dye to plasma protein. In guinea-pig airways, it has been shown that there is a significant correlation between exudation of Evan's blue and [^{125}I]-human serum albumin *in vivo* (Rogers *et al.*, 1989).

Initially it was found that abnormally high Evan's blue leakage under baseline conditions was evident in some experiments. This has also been reported by Erjefalt *et al.*, (1985), while studying tracheobronchial microvascular permeability using Evan's blue as a marker. It is likely that the elevated and spontaneous dye leakage is probably a consequence of tissue damage to the microvasculature during surgery and tracheal/nasal intubation. To account for this, approximately 5 to 6 collections and replacement of perfusion fluid were made during the equilibriation period and this resulted in a relatively stable baseline showing minimal basal leakage of dye.

The B_1 receptor agonist, [Des-Arg^9]-bradykinin, 10^{-6}M had no effect on Evan's blue leakage. This is consistent with the airway effects of kinins not being mediated via the kinin B_1 receptor in the guinea-pig (Trifilieff *et al.*, 1991) or in the human nasal airway (Rajakulasingam *et al.*, 1991; chapter 4).

Administration of bradykinin to the perfusion circuit generally resulted in an increase in

Evan's blue leakage, although this was highly variable and not dose-related. Bradykinin, 1nM induced greater dye leakage than doses of 0.1μM and 1μM. This was not expected, since a dose-related increase in vascular permeability has previously been shown in response to nasal bradykinin challenge (Proud *et al.*, 1988; chapter 4).

The data could be interpreted as simply that the model is unreliable and spontaneous leakage occured as a result of tissue damage, leading to spurious results. However, if this was the case, then it is unlikely that any reproducibility in the results would be apparent and clearly this is not the case. The baseline is reasonably stable once an initial equilibration has been allowed and all doses of bradykinin induced dye leakage over a characteristic time-course for kinin-induced changes in nasal vascular permeability. It is therefore possible that the higher doses of bradykinin, 0.1μM and 1μM, induced a smaller effect on Evan's blue dye leakage due to a physiological action of this peptide. Bradykinin is known to cause vasodilatation (Regoli & Barabe, 1980) and if the higher doses used in this study significantly affect the tone of the vasculature and cause dilation of the vessels, then it is possible that a local decrease in blood pressure is induced and as a consequence, a possible decrease in plasma exudation and dye leakage may occur. This would not necessarily be a reflection of the state of permeability of the vessels. However, the studies described in this thesis on bradykinin challenge in the human nasal airway provided no evidence of this.

Despite the possible explanations for this non dose-related effect, the current model obviously requires modification. It has been argued that such a technique where the nasal cavities are perfused with liquid inplace of air, is non-physiological and this may induce non-specific effects, which could influence the experimental results (Misawa, 1988). However, this method of assessing the response to kinin challenge by measuring vascular permeability is preferable to alternatives where kinin responses are studies by measuring nasal resistance. Such methods (Misawa & Iwamura, 1990; Mizuno *et al.*, 1991) involve kinins being administered locally and intranasal pressure is measured, as an index of nasal resistance to airflow. The problem with this is that the kinin-induced nasal secretions block the nasal passages and there is no way of clearing them. Whereas, in the model described here, a constant saline perfusion eliminates this problem.

It can be concluded that an experimental model facilitating the quantitative determination of vascular permeability in the nasal mucosa is of potential use, but that the model described here requires modification. The most important change needed is a circuit modification to avoid tissue damage during nasal intubation. This may be achieved by creating a circuit where tubing does not actually enter the nasal passages, but could be fixed by a suction mechanism over the entire external nasal area. Tissue damage would thus be minimised and abnormally high and spontaneous plasma leakage, which has been the major problem with this model, would be avoided. If this modification could be successfully made, then this model, in principle, could be applied to kinin and antigen challenge investigations. If, however, the explanation given for the lack of dose-related effects of bradykinin is correct, it is difficult to see how kinin-induced changes in blood pressure can be overcome.

SUMMARY

- The aim of the experiments described here was to attempt to develop a guinea-pig model for quantifying vascular permeability in the nasal mucosa.

- Addition of the B_1 agonist, [Des-Arg^9]-bradykinin to the nasal perfusion circuit had no effect on Evan's blue leakage.

- Bradykinin, 10nM, 0.1μM and 1μM caused increases in vascular permeability as quantified by the increase in Evan's blue measured in the perfusate.

- Bradykinin, 10nM induced a significant and maximum Evan's blue leakage at 20 minutes after addition to the perfusion circuit.

- Bradykinin 0.1μM and 1μM caused Evan's blue leakage, less in magnitude than that induced by bradykinin, 10nM.

- The model is of potential use, but requires further modification to avoid tissue damage during nasal intubation, which may be the cause of spurious results.

CHAPTER 9

General discussion and future studies

This thesis describes studies which were designed to investigate the actions of platelet-activating factor (PAF) and kinins in the human nasal airway. The results obtained indicate that both these mediators may have a role in the pathophysiology of allergic rhinitis.

The role of kinins in the nasal airway has been a major focus of this thesis. Exogenous bradykinin can mimic nasal blockage which occurs during the nasal allergic reaction and appears to be mediated via a direct receptor-mediated mechanism, involving kinin B_2 receptors, as currently classified. Evidence of a histamine, H_1 receptor-sensitive component in the response to bradykinin has also been discussed. This is likely to be a non-receptor-mediated action of this peptide, although further studies investigating kinin-mediated actions on human nasal mast cells would be useful to clarify this. The data described on the action of bradykinin in normal individuals prompted studies to investigate kinin involvement in the nasal response to antigen provocation in patients with perennial allergic rhinitis. These studies showed that a large part of the nasal allergic response to house-dust mite antigen could be attributed to bradykinin, but most interestingly, during these studies, it became apparent that the pathophysiology of perennial and seasonal allergic rhinitis may differ. Clearly this is of importance clinically, and also experimentally, since current models and reports regarding allergic rhinitis make no distinction, with regard to pathology between the two types. If there is a difference in pathology, then this needs to be addressed and clarified. It would be necessary to repeat the studies described in chapter 5 of this thesis using patients with seasonal allergic rhinitis and evaluate the effect of the same bradykinin antagonist which was found effective in attenuating the nasal response to house-dust mite in patients exhibiting allergy to this antigen.

Recent evidence suggests that the action of bradykinin in the nasal airway of patients with allergic rhinitis, may be modified at the receptor level. A study investigating kinin receptor regulation on human synovial cells found that the receptors were upregulated by pretreatment with IL-1(Bathon *et al.*, 1992). The authors interpreted this as an effect which may possibly occur in inflammatory reactions. Therefore, *in vivo*, there is the possibility that the effect of kinins are not only dependent on factors regulating kinin generation and the influence of other mediators, but also on receptor number and its regulation. Future studies, in addition to the *in vivo* functional type, could investigate kinin receptor expression in the normal and inflammed nasal mucosa by evaluating binding of [^{3}H]-bradykinin by autoradiography. Additionally, quantitative mRNA analysis could also be performed as a measure of receptor regulation at the transcriptional level.

The studies described on the action of PAF indicate that it can produce a non-specific nasal hyperresponsiveness, similar to that which occurs in allergic rhinitis. It was shown that the hyperresponsiveness was accompanied by increased levels of eosinophil cationic protein in nasal lavage and that vitamin E inhibited both the PAF-induced hyperresponsiveness and eosinophil activation. This suggests that free radical production induced by PAF may contribute to the effects of PAF in the nasal airway, and raises the question of whether vitamin E may have therapeutic benefit in patients with allergic rhinitis. Further studies are needed to address this, by studying the effect of vitamin E treatment, prior to, and during the pollen season in patients with seasonal allergic rhinitis. In addition, studies directed at the characteristics of the PAF receptor would be useful. The possible heterogeneity of the receptor needs to be clarified together with the effect of selective PAF receptor antagonists on the nasal response. Additionally, it would also be interesting to investigate whether there is a hypersensivity to PAF itself, displayed by cells which respond to this lipid. It is possible that the airways of individuals with allergic rhinitis respond in an exaggerated manner not only to allergen but also to PAF generated in the airway, resulting in augmented PAF-induced responses, as compared with normal subjects. Such a hypersensitivty mechanism has already been demonstrated in human platelets taken from diabetic subjects (Shukla *et al.*, 1992) and has been attributed to an alteration in phospholipid turnover mechanism. In the airways, receptor number and phospholipid turnover are both factors which could easily be modified in allergic

individuals and a study to investigate this would be an interesting extension to the currently documented effects and mechanisms of PAF in the airway.

Considering the studies described here and the literature available, it is quite clear that the effects *in vivo* of kinins and PAF occur via their direct receptor-mediated actions and also via interaction with other inflammatory mediators, documented to be involved in the nasal allergic response. For example, bradykinin-induced microvascular leakage in an animal model of airway inflammation involves eicosanoids and PAF, since WEB 2086, a PAF antagonist, and indomethacin inhibit the effect of bradykinin (Rogers *et al.*, 1990). This stresses the importance and usefulness of a human model in which the pharmacology of allergic rhinitis can be investigated *in vivo*, and clearly more studies could be performed to further elucidate the actions of many inflammatory mediators in the various forms of allergic rhinitis. However, it is unlikely that an understanding of kinin receptor type(s) and physiological responses generated by kinin-receptor interaction, will be gained solely from functional studies based on the order of potency of agonists, and by the use of antagonists, on isolated tissue or *in vivo*, since this relies on their selectivities. At present, selective compounds to distinguish between proposed B_2 receptor subtypes or which are selective for the putative B_3 receptor are not available. To resolve the issue of kinin receptor classification in human tissue, a molecular biological approach probably needs to be applied.

In 1991 and 1992, the first cloning studies of the kinin B_2 were reported. The first two cDNA clones, each encoding a functional B_2 receptor, were isolated from rat uterus (McEachern *et al.*, 1991) and a human lung fibroblast cell line (Hess *et al.*, 1992). The two receptors exhibit 81% homology in amino acid sequence with each other, showing a striking difference in terms of a two amino acid deletion at the N-terminal extracellular region of the human receptor. The significance of this remains to be determined, but it now clear that there are differences in pharmacology between kinin receptors from different species. This raises the question of, how relevant are the pharmacological studies describing a putative B_3 receptor in the guinea-pig large airways to the human situation? This question needs to be addressed by further studies with the cloned human B_2 receptor. Investigations with the human receptor directed at the nature of the ligand-

receptor interaction at the molecular level, are probably the way forward to a better understanding of the means by which kinins generate responses and to the development of improved kinin receptor antagonists which may serve a useful purpose in functional pharmacology and may assist in the discovery of therapeutic compounds which act through the B_2 receptor.

In conclusion, it would appear that allergic rhinitis is a condition which results from the actions of multiple inflammatory mediators following the initial IgE-mediated response. It is quite likely that the precise contributions of the various mediators in precipitating the nasal response vary from patient to patient and is probably dependent on the state of activation of the inflammatory cells involved. In addition there is evidence to suggest that the allergic reaction varies with the allergen(s) responsible and the atopic status of the individual. If this is so, then clinically, it makes treatment strategies somewhat difficult. It may possibly be speculated that the optimum therapy would be a combination of selective antagonists, which may be directed at a particular part of the cascade of inflammation. The model described in this thesis could usefully be applied to further investigations, perhaps regarding interactions between mediators because undoubtedly the study of single mediator actions may be masking synergistic interactions which could play an important role *in vivo*.

References

AITKEN, R.C.B. (1969). Measurement of feelings using visual analogue scales. Proc. Royal Soc. Med., 62, 989-993.

AKAIKE, T., ANDO, M., ODA, T., DOI, T., IJIRI, S., ARAKI, S. & MAEDA, H. (1990). Dependence on O_2^- generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. J. Clin. Invest., 85, 739-745.

ALBERT, D.H. & SNYDER, F. (1983). Biosynthesis of 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine (platelet activating factor) from 1-alkyl-2-acyl-sn-glycero-3-phosphocholine by rat alveolar macrophages. Phospholipase A_2 and acetyl-transferase activities during phagocytosis and ionophore stimulation. J. Biol. Chem. 25, 97-102.

AKBARY, M.A. & BENDER, N. (1993). Efficacy and safety of Hoe 140 (a bradykinin antagonist) in patients with mild-to-moderate seasonal allergic rhinitis. In Proceedings of Kinins 93 Brazil, Abstract 22.02.

ANDERSSON, M., VON KOGERER, B., ANDERSSON, P. & PIPKORN, U. (1987). Allergen-induced nasal hyperreactivity appears unrelated to the size of the nasal and dermal immediate allergic reaction. Allergy, 42, 631-637.

ANDERSSON, M. & PIPKORN, U. (1988). The effect of platelet activating factor on nasal hypersensitivity. Eur. J. Clin. Pharmacol. 35, 231-235.

ANDERSSON, M., ANDERSSON, P. & PIPKORN, U. (1989a). Allergen-induced specific and non-specific nasal reactions. Acta Otolaryngol. 107, 270-7.

ANDERSSON, M., ANDERSSON, P., VENGE, P. & PIPKORN, U. (1989b). Eosinophils and eosinophil cationic protein in nasal lavages in allergen-induced hyperresponsiveness: effects of topical glucocorticosteroid treatment. Allergy, 44, 342-348.

ANDERSSON, M. & PIPKORN, U. (1990). Nasal hyperreactivity. In: Rhinitis and Asthma: similarities and differences. Eds., Mygind, N., Pipkorn, U. & Dahl, R. Munksgaard, Copenhagen.

ARCHER, C.B., MACDONALD, D.M., MORLEY, J., PAGE, C.P., PAUL, W. & SANJAR, S. (1985). Effects of serum albumin, indomethacin and histamine H_1-antagonists on paf-acether-induced inflammatory responses in the skin of experimental animals and man. Br. J. Pharmacol. 85, 109-113.

AYARS, G.H., ALTMAN, L.C., McMANUS, M.M., AGOSTI, J.M., BAKER, C., LUCHTEL, D.L., LOEGERING, D.A. & GLEICH, G.J. (1989). Injurious effect of the eosinophil peroxidase-halide system and major basic protein in the human nasal epithelium *in vitro*. Am. Rev. Respir. Dis. 140, 125-131.

BACON, J.R., MCLEEN, J.A., MATHEWS, K.P. & BANAS, J.M. (1981). Priming of the nasal mucosa by ragweed extract or by an irritant (ammonia). J. Allergy Clin. Immunol. 67, 111-116.

BARABE, J., BABIUK, C. & REGOLI, D. (1982). Binding of [^{3}H]des-Arg^9-BK to rabbit anterior mesenteric vein. Can. J. Physiol. Pharmacol. 60, 1551-1555.

BARANIUK, J.N., CASTELLINO, S., LUNDGREN, J.D., GOFF, J., MULLOL, J., MERIDA, M., SHELHAMER, J.H. & KALINER, M.A. (1990a). Neuropeptide Y (NPY) in human nasal mucosa. Am. J. Resp. Cell Molec. Biol. 3,165-174.

BARANIUK, J.N., LUNDGREN, J.D., GOFF, J., MULLOL, J., CASTELLINO, S., MERIDA, M., SHELHAMER, J.H. & KALINER, M.A. (1990b). Calcitonin gene-related peptide in human nasal mucosa. Am. J. Physiol. 258, L81-88.

BARANIUK, J.N., LUNDGREN, J., MIZOGUCHI, H., PEDEN, D., GAWIN, A., MERIDA, M., SHELHOME, J. & KALINER, M.A. (1990c). Bradykinin and respiratory mucus membranes: analysis of BK binding site distribution and secretory responses *in vitro* and *in vivo*. Am. Rev. Respir. Dis. 141, 706-714.

BARBARO, J.F. & ZVAILER, N.J. (1966). Antigen-induced histamine release from platelets of rabbits producing homologous PCA antibody. Proc. Soc. Exp. Biol. Med. 122, 1245-1247.

BARNES, P.J. (1986). Asthma as an axon reflex. Lancet 1, 242-245.

BARNES, P.J., CHUNG, K.F., EVANS, T.W. & ROGERS, D.F. (1986). Increase in airway vascular permeability induced by platelet-activating factor: effect of platelet depletion and specific antagonism. Br. J. Pharmacol. 89, 764P.

BARNES, P.J. & COSTELLO, J.F. (1987). Airway hyperresponsiveness and inflammation. Br. Med. Bull. 43, 445-459.

BARNES, P.J., BARANIUK, J.N. & BELVISI, M.G. (1991). Neuropeptides in the respiratory tract. Part I. Am. Rev. Respir. Dis. 144, 1187-1198.

BARNES, P.J., BARANIUK, J.N. & BELVISI, M.G. (1991). Neuropeptides in the respiratory tract. Part II. Am. Rev. Respir. Dis. 144, 1391-1399.

BARRETT, K.E. & PEARCE, F.L. (1993). Mast cell heterogeneity. In: Immunopharmacology. Mast cells and basophils. p 29-42. Ed. J.C. Foreman. Academic press, London.

BASCOM, R., PIPKORN, U., LICHTENSTEIN, L.M. & NACLERIO, R.M. (1988a). The influx of inflammatory cells into nasal washings during the late response to antigen-challenge: effect of systemic steroid treatment. Am. Rev. Respir. Dis. 138, 406-412.

BASCOM, R., WACHS, M., NACLERIO, R.M., PIPKORN, U., GALLI, S. & LICHTENSTEIN, L.M. (1988b). Basophil influx occurs after nasal antigen challenge: effect of corticosteroid pretreatment. J. Allergy Clin. Immunol. 81, 580-589.

BATHON, J.M., MANNING, D.C., GOLDMAN, D.W., TOWNS, M.C. & PROUD, D. (1992). Characterization of kinin receptors on human synovial cells and upregulation of receptor number by interleukin-1. J. Pharmacol. Exp. Ther. 260, 384-392.

BAUMGARTEN, C.R., TOGIAS, A.G., NACLERIO, R.M., LICHTENSTEIN, L.M., NORMAN, P.S. & PROUD, D. (1985). Influx of kininogens into nasal secretions after antigen

challenge of allergic individuals. J. Clin. Invest. 76, 191-197.

BAUMGARTEN, C.R., NICHOLS, R.C., NACLERIO, R.M. & PROUD, D. (1986). Concentrations of glandular kallikrein in human nasal secretions increase during experimentally-induced allergic rhinitis. J. Immunol. 137, 1323-1328.

BENHAMON, M., NINIO, F., SALEM, P., HIEBLAT, C., BESSAN, G., PITTON, C., LIM, F.T. & MENCIA-HUERTA, J.M. (1986). Decrease in IgE Fc expression on mouse bone marrow derived mast cells and inhibition of PAF-acether formation and beta-hexose aminidase release by dexamethasone. J. Immunol. 136, 1385-1392.

BENVENISTE, J., HENSON, P.M. & COCHRANE, C.G. (1972). Leucocyte dependent histamine release from rabbit platelets: The role of IgE, basophils and a platelet activating factor. J. Exp. Med., 136, 1356-1377.

BENVENISTE, J., TENCE, M., VARENNE, P., BIDAULT, J., BOULLET, C. & POLONSKY, J. (1979). Semi-synthese et structure propose du facteur activant les plaquettes (PAF): PAF-acether, un alkyl ether analogue de la phosphatidylcholine. C. R. Acad. Sci. (Paris), 289, 1037-1040.

BENVENISTE, J. (1987). A role for platelet activating factor in bronchial hyperreactivity. Am. Rev. Respir. Dis. 135, S2-S4.

BENYON, R.C., LOWMAN, M.A. & CHURCH, M.K. (1987). Human skin mast cells: their dispersion, purification and secretory characterization. J. Immunol. 138, 861-867.

BISGAARD, H., OLSSON, P. & BENDE, M. (1986). Effect of leukotriene D_4 on nasal mucosal blood flow, nasal airway resistance and nasal secretion in humans. Clin. Allergy 16, 289-297.

BLACKLEY, C.H. (1873). Experimental researches on the causes and nature of catarrhus aestivus. London: Bailliere, Tindall, Cox.

BLANK, M.L., SNYDER, F., BYERS, L.W., BROOKS, B. & MUIRHEAD, E.E. (1979). Antihypertensive activity of an alkyl ether analog of phosphotidylcholine. Biochem. Biophys. Res. Commun. 90, 1194-1200.

BORUM, P.(1979). Intranasal methacholine challenge: a test for the measurement of nasal reactivty. J. Allergy Clin.Immunol. 63, 253-257.

BORUM, P., GRONBERG, H., BROFELDT, S., MYGIND, N. (1983). Nasal reactivity in rhinitis. Eur. J. Respir. Dis. 64, 65-71.

BOUSHEY, H.A., HOLTZMAN, M.J., SHELLER, J.R. & NADEL, J.A. (1980). State of the Art. Bronchial hyperreactivity. Am. Rev. Respir. Dis. 121, 389-413.

BOUSHEY, H.A. & HOLTZMAN, M.J. (1985). Experimental airway inflammation and hyperreactivity. Am. Rev. Respir. Dis. 131, 312-313.

BOUSQUET, J., LEBEL, B., CHANAL, I., MOREL, A., MICHEL, F.B. (1988). Antiallergic activity of H_1-receptor antagonists assessed by nasal challenge. J. Allergy Clin. Immunol., 82, 881-887.

BOUTHILLIER, J., DeBLOIS, D. & MARCEAU, F. (1987). Studies on the induction of pharmacological responses to des-Arg^9-bradykinin *in vitro* and *in vivo*. Br. J. Pharmacol. 92, 257-264.

BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). CGRP is a potent vasodilator. Nature 313, 54-56.

BRAQUET, P. & GODFROID, J.J. (1987). Conformational properties of the PAF-acether receptor on platelets based on structure-activity studies. In: Platelet activating factor and related lipid mediators. Ed. by Snyder, F. New York, Plenum. p.153-190.

BRAQUET,P., TOUQUI, L., SHEN, T.Y. & VARGAFTIG, B.B. (1987b) Perspectives in platelet-activating factor research. Pharmacol.Rev. 39, 97-145.

BRAUNSTEIN, G., MALAQUIN, F., FAJAC, I., MELAC, M. & FROSSARD, N. (1992). Inhibition of histamine-induced nasal obstruction by cetirizine in allergic rhinitis. Br. J. Clin. Pharmacol. 33, 445-448.

BRITTON, M.G., EMPEY, D.W., JOHN, G.C., McDONNELL, K.A. & HUGHES, D.T.D. (1978). Histamine challenge and anterior nasal rhinometry: their use in the assessment of pseudoephedrine and tripolidine. Br. J. Clin. Pharmacol., 6, 51-58.

BROWN, I.G., ZAMEL, N. & HOFFSTEIN, V. (1986). Pharyngeal cross-sectional area in normal men and women. J. Appl. Physiol., 61, 890-895.

BRUNTON, L.L., WIKLUND, R.A., VAN ARSDALE, P.M. & GILMAN, A.G. (1976). Binding of [^{3}H]prostaglandin E_1 to putative receptors linked to adenylate cyclase of cultured cell clones. J. Biol. Chem. 251, 3037-3044.

BUEB, J.L., MOUSLI, M., LANDRY, Y. & BRONNER, C. (1990a). A pertussis toxin-sensitive G-protein is required to induce histamine release from rat peritoneal mast cells by bradykinin. Agents Actions 30, 98-101.

BUEB, J.L., MOUSLI, M., BRONNER, C., ROUOT, B. & LANDRY, Y. (1990b). Activation of G_i-like proteins, a receptor-independent effect of kinins in mast cells. Molec. Pharmacol. 38, 816-822.

BURCH, R.M., FARMER, S.G. & STERANKA, L. (1990). Bradykinin receptor antagonists. Med. Res. Rev. 10, 237-269.

CASALS-STENZEL, J. & WEBER, K.H. (1987). Triazolobenzodiazepines: dissociation of their PAF (platelet activating factor) antagonistic and CNS activity. Br. J. Pharmacol. 90, 139-146.

CAUGHEY, G.H. (1989). Roles of mast cell tryptase and chymase in airway function. Am. J. Physiol. 257, L39-46.

CAUNA, N. (1982). Blood and nerve supply of the nasal lining. In: Proctor D, Andersen I. eds. The Nose, Elsevier.

CHAPMAN, I.D., BOUBEKEUR, K. & MORLEY, J. (1991). Airway eosinophilia and airway hyperreactivity are parallel rather than sequential events in the guinea-pig. Agents Actions 34,

445-456.

CHIGNARD, M., Le COUEDIC, J.P., ANDERSSON, P. & BRANGE, C. (1986). Use of steroidal antiinflammatory drugs provides further evidence for a potential role of PAF acether in bronchial anaphylaxis. Int. Arch. Allergy Appl. Immunol. 81, 184-185.

CHRISTIANSEN, S.C., PROUD, D., SARNOFF, R.B., JUERGENS, U., COCHRANE, C.G. & ZURAW, B.L. (1992). Elevation of tissue kallikrein and kinin in the airways of asthmatic subjects after endobronchial allergen challenge. Am. Rev. Respir. Dis. 145, 900-905.

CHUNG, K.F., AIZCIWA, H., LEIKAUF, G.D., UEKI, I.F., EVANS, T.W. & NADEL, J.A. (1986). Airway hyperresponsiveness induced by platelet-activating factor: role of thromboxane generation. J. Pharmacol. Exp. Ther., 236, 580-584.

CHUNG, K.F., DENT, G., McCUSKER, M., GUINOT, P., PAGE, C.P. & BARNES, P.J. (1987). Effect of a gingkolide mixture (BN52063) in antagonising skin and platelet responses to platelet activating factor in man. Lancet 1, 248-251.

CHURCH, M.K. & WARNER, J.O. (1985). Sodium cromoglycate and related drugs. Clin. Allergy 15, 311-20.

CHURCHILL, L., PONGRACIC, J.A., REYNOLDS, C.J., NACLERIO, R.M. & PROUD, D. (1991). Pharmacology of nasal provocation with bradykinin: Studies of tachyphylaxis, cyclooxygenase inhibition, α-adrenergic stimulation and receptor subtype. Int. Arch. Allergy Appl. Immunol. 95, 322-331.

CLEMENT, P.A.R. (1984). Committee report on standardization of rhinomanometry. Rhinology 22, 151-155.

COCKCROFT, D.W. (1983). Mechanism of perennial allergic asthma. Lancet 2, 253-256.

COHAN, V.L., MACGLASHAN, D.W., WARNER, J.A., LICHTENSTEIN, L.M. & PROUD, D. (1991). Mechanisms of mediator release from human skin mast cells upon stimulation by the bradykinin analog, [D-Arg^0- Hyp^3-DPhe^7]-bradykinin. Biochem. Pharmacol. 41, 293-300.

CONNELL, J. (1969). Quantitative intranasal pollen challenge: the priming effect in allergic rhinitis. J. Allergy 43, 33-38.

COOKSON, W.O.C.M., SHARP, P.A., FAUX, J.A. & HOPKIN, J.M. (1989). Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet I, 1292-1295.

CORRADO, O.J., GOULD, C.A., KASSAB, J.Y. & DAVIES, R.J. (1986). Nasal response of rhinitic and non-rhinitic subjects to histamine and methacholine: a comparative study. Thorax 41, 863-868.

CORRADO, O.J., OLLIER, S., PHILLIPS, M.J., THOMAS, J.M. & DAVIES, R.J. (1987). Histamine and allergen induced changes in nasal airways resistance measured by anterior rhinomanometry: reproducibility of the technique and the effect of topically administered antihistaminic and anti-allergic drugs. Br. J. Clin. Pharmacol., 24, 283-292.

COUTURE, R., MIZRAHI, J., REGOLI, D. & DEVROEDE, G. (1981). Peptides and the

human colon: an *in vitro* pharmacological study. Can. J. Physiol. Pharmacol. 59, 957-964.

COYLE, A.J., SPINA, D. & PAGE, C.P. (1990). PAF-induced bronchial hyperresponsiveness in the rabbit: contribution of platelets and airway smooth muscle. Br. J. Pharmacol. 101, 31-38.

COYLE, A.J., BURCH, R.M. & IRVIN, C.G. (1992). A synthetic cationic protein induces airway hyperresponsiveness *in vivo* dependent on the generation of bradykinin. Am. Rev. Respir. Dis. 145, A38.

CRETICOS, P.S., PETERS, S.P., ADKINSON, N.F., NACLERIO, R.M., HAYES, E.C., NORMAN, P.S. & LICHTENSTEIN, L.M. (1984). Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. New Eng. J. Med. 310, 1626-30.

CUSS, F.M., DIXON, C.M.S. & BARNES, P.J. (1986). Effects of inhaled platelet-activating factor on pulmonary function and bronchial responsiveness in man. Lancet, 2, 189-192.

DALE, H.H. & LAIDLAW, P.P. (1911). The physiologic action of beta-imidazolethylamine. J. Physiol. 41, 318-344.

DE CHATELET, L.R., SHIRLEY, P.S., McPHAIL, L.C., HUNTLEY, C.C., MUSS, H.B. & BASS, D.A. (1977). Oxidation metabolism of the human eosinophil. Blood 50, 526-535.

DEMOPOULOS, C.A., PINCKARD, R.N. & HANAHAN, D.J. (1979). Platelet activating factor: evidence for 1-o-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). J. Biol. Chem. 254, 9355-9358.

DEVILLIER, P., RENOUX, M., GIROUD, J.P. & REGOLI, D. (1985). Peptides and histamine release from rat peritoneal mast cells. Eur. J. Pharmacol. 117, 89-96.

DEVILLIER, P., RENOUX, M., DRAPEAU, G. & REGOLI, D. (1988). Histamine release from rat peritoneal mast cells by kinin antagonists. Eur. J. Pharmacol. 149, 137-140.

DEVILLIER, P., DRAPEAU, G., RENOUX, M. & REGOLI, D. (1989). Role of the N-terminal arginine in the histamine-releasing activity of substance P, bradykinin and related peptides. Eur. J. Pharmacol. 168, 53-60.

DISHOECK, H.A.E.van & LIER, L.A.J.van. (1960). Ausserliche Reize bei Rhinopathia vasomoria allergica und non-allergica. Acta Otolaryngol. 51, 275-83.

DOLOVICH, J., BACK, N. & ARBESMAN, C.E. (1970). Kinin-like activity in nasal secretions of allergic patients. Int. Arch. Allergy 38, 337-344.

DOLOVICH, J., KENNEDY, R.N., VICKERSON, F. & KAZIM, F. (1987). Control of the hypersecretion of vasomotor rhinitis by topical ipratropium bromide. J. Allergy Clin. Immunol. 80, 274-8.

DRAZEN, J.M., AUSTEN, K.F. & LEWIS, R.A. (1980). Comparative airway and vascular activities of leukotrienes C-1 and D *in vivo* and *in vitro*. Proc. Natl. Acad. Sci. USA. 77,4354-4358.

DRETTNER, B. (1963). Blood vessel reactions in the nasal mucosa. Int. Rhinol. 1, 40-45.

DRUCE, H.W., WRIGHT, R.H., KOSSOF, D. & KALINER, M.A. (1985). Cholinergic nasal hyperreactivity in atopic subjects. J. Allergy Clin. Immunol. 76, 445-452.

D'URZO, A.D., LAWSON, V.G., VASSAL, K.P., REBUCK, A.S. & HOFFSTEIN, V. (1987). Airway area by acoustic response measurements and computed axial tomography. Am. Rev. Respir. Dis., 135, 392-395.

ERDOS, E.G. (1979). Kininases. In: Erdos, E.G. ed. Handbook of experimental pharmacology. Bradykinin, kallidin and kallikrein. New York: Springer-Verlag. p.427-487.

ERDOS, E.G. & SKIDGEL, R.A. (1989). Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. FASEB J. 3, 145-151.

ERJEFALT, I.A.L., G-WAGNER, Z., STRAND, S.E. & PERSSON, C.G.A. (1985). A method for studies of tracheobronchial microvascular permeability to macromolecules. J. Pharmacol. Methods 14, 275-283.

EVANS, T.W., CHUNG, K.F., ROGERS, D.F. & BARNES, P.J. (1987). Effect of platelet-activating factor on airway vascular permeability: possible mechanisms. J. Appl. Physiol. 63, 479-484.

FARMER, S.G., BURCH, R.M., MEEKER, S.A. & WILKINS, D.E. (1989). Evidence for a pulmonary B_3 receptor. Mol. Pharmacol. 36, 1-8.

FARMER, S.G. & BURCH, R.M. (1991a). The pharmacology of bradykinin receptors. In: Bradykinin antagonists. Burch, R.M. ed. Marcel Dekker, Inc., New York. p. 1-31.

FARMER, S.G., BURCH, R.M., KYLE, D.J., MARTIN, J.A., MEEKER,S.N. & TOGO, J. (1991b). D-Arg[Hyp^3,Thi^5,D-Tic^7,Tic^8]-bradykinin, a potent antagonist of smooth muscle BK_2 and BK_3 receptors. Br. J. Pharmacol. 102, 785-787.

FARMER, S.G., ENSOR, J.E., BURCH, R.M. (1991c). Evidence that cultured airway smooth muscle cells contain bradykinin B_2 and B_3 receptors. Am. J. Respir. Cell. Mol. Biol., 4, 273-277.

FARMER, S.G., WILKINS, D.E., MEEKER, S A., SEEDS, E.A.M. & PAGE, C.P. (1992). Effects of bradykinin receptor antagonists on antigen-induced respiratory distress, airway hyperresponsiveness and eosinophilia in guinea-pigs. Br. J. Pharmacol. 107, 653-659.

FEWTRELL, C.M.S., FOREMAN, J.C., JORDAN, C.C., OEHME, P., RENNER, H. & STEWART, J.M. (1982). The effects of substance P on histamine and 5-hydroxytryptamine release in the rat. J. Physiol. 330, 393-411.

FIELD, J.L., HALL, J.M. & MORTON, I.K.M. (1992) Bradykinin receptors in the guinea-pig taenia caeci are similar to proposed B_3 receptors in the guinea-pig trachea and are blocked by HOE 140. Br. J. Pharmacol. 105, 293-296.

FINKELMAN, F.D., KATONA, I.M., URBAN, J.F., SNAPPER, C.M., OHARA, J. & PAUL, W.E. (1986). Suppression of *in vivo* polyclonal IgE responses by monoclonal antibody to the lymphocyte B-cell stimulating factor 1. Proc. Natl Acad. Sci. U.S.A. 83, 9675-9678.

FLAVAHAN, N.A., SLIFMAN, N.R., GLEICH, G.J. & VANHOUTTE, P.M. (1988). Human eosinophil major basic protein causes hyperreactivity of respiratory smooth muscle. Role of the epithelium. Am. Rev. Respir. Dis. 138, 685-688.

FLEMING, D.M. & CROMBIE, D.L. (1987). Prevalence of asthma and hayfever in England and Wales. Br. Med. J. 294, 279-283.

FOREMAN, J.C., JORDAN, C.C. & PIOTROWSKI, W. (1982). Interaction of neurotensin with the substance P receptor mediating histamine release from rat mast cells and the flare in human skin. Br. J. Pharmacol. 77, 531-539.

FRAGATA., M. & BELLEMARE, F. (1980). Model of singlet oxygen scavenging by α-tocopherol in biomembranes. Chem. Phys. Lipids, 27, 93-99.

FRANCEL, P.C., KEEFER, J.F. & DAWSON, G. (1989). Bradykinin analogs antagonize bradykinin-induced second messenger production in a sensory neuron cell line. Mol. Pharmacol. 35, 34-38.

FREELAND, H.S., PIPKORN, U., SCHLEIMER, R.P., BASCOM, R., LICHTENSTEIN, L.M., NACLERIO, R.M. & PETERS, S.P. (1989). Leukotriene B_4 as a mediator of early and late reactions to antigen in humans. The effect of systemic glucocorticoid treatment *in vivo*. J.Allergy Clin. Immunol. 83, 634-42.

FRIGAS, E., LOEGERING, D.A. & GLEICH, G.J. (1980). Cytotoxic effects of the guinea-pig eosinophil major basic protein on tracheal epithelium. Lab. Invest. 42, 35-43.

FRIGAS, E. & GLEICH, F.J. (1986). The eosinophil and the pathophysiology of asthma. J. Allergy Clin. Immunol. 77, 527-537.

FULLER, R.W., DIXON, C.M.S., DOLLERY, C.T. & BARNES, P.J. (1986). Prostaglandin D_2 potentiates airway responses to histamine and methacholine. Am. Rev. Respir. Dis. 133, 252-254.

GAFFEY, M., GWALTHEY, J., SASTRE, A., DRESSLER, W., SORRENTINO, J. & HAYDEN, F. (1987). Intranasally and orally administered anti-histamine treatment of experimental rhinovirus colds. Am. Rev. Respir. Dis. 136,. 556-560.

GEPPETTI, P., BERTRAND, C. & NADEL, J.A. (1994). The role of bradykinin and sensory nerves in the vascular extravasation evoked by antigen in guinea-pig trachea. Br. J. Pharmacol. 111, 50P.

GIBSON, P.G., DENBURG, J., DOLOVICH, J., RAMSDALE, E.H. & HARGREAVE, F.E. (1989). Chronic cough: eosinophilic bronchitis without asthma. Lancet 1, 1346-1348.

GLEESON, M.J., YOULTEN, L.J.F., SHELTON, D.M., SIODLAK, M.Z., EISER, N.M. & WENGRAF, C.L. (1986). Assessment of nasal airway patency: a comparison of four methods. Clin.Otolaryngol., 11, 99-107.

GODFROID, J.J., HEYMANS, F., MICHEL, E., REDEUILH, C., STEINER,E. & BENVENISTE, J. (1980). Platelet activating factor (PAF-acether): total synthesis of 1-o-octadecyl 2-o-acetyl sn-glycero-3-phosphocholine. FEBS Lett. 116,161-164.

GOMEZ, E., CORRADO, O.J., BALDWIN, D.L., SWANSTON, A.R. & DAVIES, R.J. (1986). Direct *in vivo* evidence for mast cell degranulation during allergen induced reactions in man. J. Allergy Clin. Immunol. 78, 637-645.

GOMEZ, E., CLAGUE, J.E., GATLAND, D. & DAVIES, R.J. (1988). Effect of topical corticosteroids on seasonally induced increases in nasal mast cells. Br. Med. J. 296, 1572-1573.

GRONBERG, H., BORUM, P. & MYGIND, N. (1986). Histamine and methacholine do not increase nasal reactivity. Clin. Allergy 16, 597-602.

HAMILTON, L.H. (1979). Nasal airway resistance: the measurement and regulation. Physiologist 22, 43-49.

HEMLER, M.E., COOK, H.W. & LANDS, W.E. (1979). Prostaglandin synthesis can be triggered by lipid peroxides. Arch. Biochem. Biophys. 173, 340-345.

HENOCQ, E. & VARGAFTIG, B.B. (1986). Accumulation of eosinophils in response to intracutaneous Paf-acether and allergen in man. Lancet 1, 1378-1379.

HENOCQ, E. & VARGAFTIG, B.B. (1988). Skin eosinophilia in atopic patients. J. Allergy Clin. Immunol. 81, 691-695.

HERD, C.M., DONIGI-GALE, D., SHOUPZ, T.S. & PAGE, C.P. (1992). Effect of a combined 5-lipoxygenase inhibitor and leukotriene antagonist (PF5901) on PAF-induced airway responsiveness and cellular infiltration in neonatally immunised rabbits. Br. J. Pharmacol. 107, 1108-1115.

HESS, J.F., BORKOWSKI, J.A., YOUNG, G.S., STRADER, C.D. & RANSOM, R.W. (1992). Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. Biochem. Biophys. Res. Commun. 184, 260-268.

HIGGINS, P.G., BARROW, G.I. & TYRELL, D.A.J. (1990). A study of the efficacy of the bradykinin antagonist NPC 567 in rhinovirus infection in human volunteers. Antivir. Res. 14, 339-344.

HILBERG, O., JACKSON, A.C., SWIFT, D.L. & PEDERSON, O.F. (1989). Acoustic rhinometry: evaluation of nasal cavity geometry by acoustic reflection. J. Appl. Physiol., 66, 295-303.

HILDING, A. (1972). A simple method for collecting near normal nasal secretions. Ann. Otol. Rhinol. Laryngol. 81, 422-423.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, S., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHOLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: *in vitro* studies. Br. J. Pharmacol. 102, 769-773.

HOGG, J.C. (1981) Bronchial mucosal permeability and its relationship to airway hyperreactivity. J. Allergy Clin. Immunol., 67, 421-425.

HOLMSTROM, M., SCADDING, G.K., LUND, V.J. & DARBY, Y.C. (1990). A comparison between rhinomanometry and nasal inspiratory peak flow. Rhinology 28, 191-196.

HOLTZMAN, M.J., FABBRI, L.M., O'BYRNE, P.M., GOLD, B.D., AIZAWA, H., WALTERS, E.H., ALPERT, S.E. & NADEL, J.A. (1983). Importance of airway inflammation for hyperresponsiveness induced by ozone in dogs. Am. Rev. Respir. Dis. 127, 686-690.

HONDA, Z., NAKAMURA, M., MIKI, I., MINAMI, M., WATANABE, T., SEYAMA, Y., OKADO, H., TOH, H., ITO, K., MIYAMOTO, T. & SHIMIZU, T. (1991). Cloning and functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 349, 342-346.

HORAK, F., TOTH, J., JAGER, S., BERGER, U. (1993). Effects of H_1-receptor antagonists on nasal obstruction in atopic patients. Allergy 48, 226-229.

HOWARTH, P.H. (1989). Allergic rhinitis: a rational choice of treatment. Respir. Med. 83, 179-188.

HWANG, S.B., LAM, M.H., BIFTU,T., BEATTIE, T.R. & SHEN,T.Y. (1985). Trans-2,5-Bis-(3,4,5,-trimethotyphenyl) tetrahydrofuran: an orally active specific and competitive receptor antagonist of platelet activating factor. J. Biol. Chem. 260, 15639-15645.

HWANG, S.B., LAM, M.H. & SHEN, T.Y. (1985). Specific binding sites for platelet-activating factor in human lung. Biochem. Biophys. Res. Commun. 128, 972-979.

HWANG, S.B. (1990). Specific receptors of platelet activating factor, receptor heterogeneity, and signal transduction mechanisms. J. Lipid Med. 2, 123-158.

INGELSTEDT, S. & IVSTAM, B. (1951). Study in the humidifying capacity of the nose. Acta. Otolaryngol. 39, 286-289.

ISHIZAKA, T., IWATA, M. & ISHIZAKA, K. (1985). Release of histamine and arachidonate from mouse mast cells induced by glycosylation-enhancing factor and bradykinin. J. Immunol. 134, 1880-1887.

JACKSON, A.C., BUTLER, J.P., MILLET, E.J., HOPPIN, F.G. & DAWSON, S.V. (1977). Airway geometry by analysis of acoustic pulse response measurements. J. Appl. Physiol., 43, 523-536.

JACKSON, A.C. & KREVANS, J.R. (1984). Tracheal cross-sectional areas from acoustic reflections in dogs. J. Appl. Physiol., 57, 351-353.

JOHNSON, A.R. & ERDOS, E.G. (1973). Release of histamine from mast cells by vasoactive peptides. Proc. Soc. Exp. Biol. Med. 142, 1252-1256.

JOSEPH, M., AURIAULT, C., CAPRON, A., VORNG, H. & VIENS, P. (1983). A new function for platelets: IgE-dependent killing of schistosomes. Nature 303, 810-812.

KAINER, M., WASSERMAN, S.I. & AUSTEN, K.F. (1973). Immunologic release of chemical mediators fron human nasal polyps. New Eng. J. Med. 289, 278-81.

KATO, M., TOKUYAMA, K., MORIKAWA, A., KUROUME, T. & BARNES, P.J. (1993). Platelet-activating factor-induced enhancement of superoxide anion generation in guinea-pigs. Eur. J. Pharmacol. 232,7-12.

KATSUMATA, U., MIURA, M., ICHINOSE, M., KIMURA, K., TAKAHASHI, Y., INOUE, H. & TAKISHIMA, T. (1990). Oxygen radicals produce airway constriction and hyperesponsiveness in anaesthetised cats. Am. Rev. Respir. Dis., 141, 1158-1161.

KAY, A.B., YING, S., VARNEY, V., GAGA, M., DURHAM, S.R., MOQBEL, R., WARDLAW, A.J. & HAMID, Q. (1991). Messenger RNA expression of the cytokine gene cluster, IL-3, IL-4, IL-5 and GM-CSF in allergen-induced late-phase cutaneous reactions in atopic subjects. J. Exp. Med. 173, 775-778.

KELLERMEYER, R.W. & GRAHAM, R.C. (1968). Kinins - possible physiologic and pathologic roles in man. N. Eng. J. Med. 279, 859-865.

KERN, E. (1973). Rhinomanometry. Otolaryngol. Clin. N. Am. 6, 863-874.

KLEMENTSSON, H., ANDERSSON, M., BAUMGARTEN, C.R., VENGE, P. & PIPKORN, U. (1990). Changes in non-specific nasal reactivity and eosinophil influx and activation after allergen challenge. Clin. Exp. Allergy 20, 539-49.

KLEMENTSSON, H., VENGE, P., ANDERSSON, M. & PIPKORN, U. (1991). Allergen-induced changes in nasal secretory responsiveness and eosinophil granulocytes. Acta Otolaryngol. Stockh. 111, 776-784.

KNAPP, H.R. (1990). Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor. New Eng. J. Med. 323, 1745.

KNAUER, K.A., LICHTENSTEIN, L.M., ADKINSON, N.F. & FISH, J.E. (1981). Platelet activation during antigen-induced airway reactions in asthmatic subjects. New Eng. J. Med. 304, 1404-1407.

KNOLLE, J., BREIPOHL, G., HENKE, S., WIRTH, K. & SCHOLKENS, B. (1992). New and highly potent bradykinin antagonists. Agents Actions 38, 559-564.

KONNO, A., TERADA, N. & OKAMOTO, Y. (1987). Changes of adrenergic and muscarinic cholinergic receptors in nasal mucosa in nasal allergy. J. Otorhinolaryngol. Relat. Spec. 49, 103-111.

KORNECKI, E., GARLICH, Y.H. & LENOX, R.H. (1985). Platelet activating factor-induced aggregation of human platelets specifically inhibited by triazolobenzodiazepines. Science 226, 1954-1956.

KORTEKANGAS, A. (1972). Significance of anterior and posterior technique in rhinomanometry. Acta Otolaryngol., 73, 218-221.

KROEGEL, C., YUKAWA, T., DENT, G., VENGE, P., CHUNG, K.F. & BARNES, P.J. (1989). Stimulation of degranulation from human eosinophils by platelet-activating factor. J. Immunol. 142, 3518-3526.

LAMMEK, B., WANG, Y., GAVRAS, I. & GAVRAS, H. (1990). A new highly potent antagonist of bradykinin. Peptides 11, 1041-1043.

LAMMEK, B., WANG, Y., GAVRAS, I. & GAVRAS, H. (1991). A novel bradykinin antagonist with improved properties. J. Pharm. Pharmacol. 43, 887-888.

LAWRENCE, I.D., WARNER, J.A., COHEN, V.L., LICHTENSTEIN, L.M., KAGEY-SOBOTKA, A., VAVREK, R.J., STEWART, J.M. & PROUD, D. (1989). Induction of histamine release from human skin mast cells by bradykinin analogues. Biochem. Pharmacol. 38, 227-233.

LEE, T.C., LENIHAN, D.J., MALONE, B., RODDY, L.L. & WASSERMAN, S.I. (1984). Increased biosynthesis of platelet-activating factor in activated human eosinophils. J. Biol. Chem. 259, 5526-5530.

LEVEEN, H.H. & FISHMAN, W.H. (1947). Combination of Evans blue with plasma protein: Its significance in capillary permeability studies, blood dye disappearance curves, and its use as a protein tag. Am. J. Physiol. 151, 26-33.

LEWIS, R.A., AUSTEN, K.F. & SOBERMAN, R.J. (1990). Leukotrienes and other products of the 5-lipoxygenase pathway. N. Eng. J. Med. 323, 645-55.

LI KAM WA, T.C., COOKE, E.D. & TURNER, P. (1992). Effects of selective histamine receptor antagonists on skin responses to intradermal bradykinin in healthy volunteers. J. Pharm. Pharmacol. 44, 869-872.

LINDER, A., VENGE, P. & DEUSCHL, H. (1987). Eosinophil cationic protein and myeloperoxidase in nasal secretions as markers of inflammation in allergic rhinitis. Allergy, 42, 583-590.

LINDER, A., STRANDBERGE, K. & DEUSEHL, H. (1988). Variations in histamine concentrations in nasal secretions in patients with allergic rhinitis. Allergy 43, 119-126.

LLONA, I., VAVREK, R., STEWART, J. & HUIDOBRO, J.P. (1987). Identification of pre- and post-synaptic bradykinin receptor sites in the vas deferens: evidence for structural prerequisites. J. Pharmacol. Exp. Ther. 241, 608-614.

LOPEZ, A.F., SANDERSON, C.J., GAMBLE, J.R., CAMPBELL, H.R., YOUNG, I.G. & VADAS, M.A. (1988). Recombinant human interleukin-5 is a selective activator of human eosinophil function. J. Exp. Med. 167, 219-224.

LOZEWICZ, S. & DAVIES, R.J. (1990). Inflammatory changes in the nasal and bronchial mucous membrane in allergic rhinitis and asthma. In: Rhinitis and Asthma. Mygind, N., Pipkorn, U. & Dahl, R. eds. Munksgaard, Copenhagen. p. 47-64.

LUDWIG, J.C., & PINKARD, R.N. (1987). Diversity in the chemical structures of neutrophil-derived platelet activating factors. In: New Horizons in Platelet activating factor Research; Winslow, C.M., & Lee, M.L., Eds., John Wiley & Sons, Chichester, 59.

LUNDBERG, J.M., LUNBLAD, L., MARTLING, C.R., SARIA, A., STJARNE, P. & ANGGARD, A. (1987). Coexistence of multiple peptides and classic transmitters in airway neurons: functional and pathophysiological aspects. Am. Rev. Respir. Dis. 136, S16-S22.

LUNDGREN, R., SODERBERG, M., HORSTEDT, P. & STENLING, R. (1988). Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. Eur. Respir. J. 1, 883-889.

LYNCH, J.M. & HENSON, P.M. (1986). The intracellular retention of newly synthetised

platelet-activating factor. J. Immunol. 137, 2653-2661.

MAK, J.C.W. & BARNES, P.J. (1991). Autoradiographic visualization of bradykinin receptors in human and guinea pig lung. Eur. J. Pharmacol. 194, 37-44.

MALLET, A.I., & CUNNINGHAM, F.M. (1985). Structural identification of platelet activating factor in psoriatic scale. Biochem. Biophys. Res. Commun. 126, 192-198.

MALMBERG, H. (1979). Symptoms of chronic and allergic rhinitis and occurence of nasal secretion granulocytes in university students, school children and infants. Allergy 34, 389-394.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochem. 2, 235-254.

MARCEAU, F., BARABE, J., ST-PIERRE, S. & REGOLI, D. (1980). Kinin receptors in experimental inflammation. Can. J. Physiol. Pharmacol. 58, 536-542.

MARCEAU, F., GENDREAU, M., BARABE, J., ST-PIERRE, S. & REGOLI, D. (1981a). The degradation of bradykinin (BK) and of des-Arg^9-BK in plasma. Can. J. Physiol. Pharmacol. 59, 131-138.

MARCEAU, F., KNAP, M. & REGOLI, D. (1981b). Pharmacological characterisation of the vascular permeability enhancing effects of kinins in the rabbit skin. Can. J. Physiol. Pharmacol. 59, 921-926.

MARCEAU, F., LUSSIER, D., REGOLI, D. & GIROUD, J.P. (1983). Pharmacology of kinins. Their relevance to tissue injury and inflammation. Gen. Pharmacol. 14, 209-299.

MAROM, Z., SHELHAMER, J.H., BACH, M.K., MORTON, D.R. & KALINER, M.A. (1982). Slow reacting substances, leukotrienes C_4 and D_4, increase the release of mucus from human airways *in vitro*. Am. Rev. Respir. Dis. 126, 449-51.

McBRIDE, P., BRADLEY, D. & KALINER, M. (1988). Evaluation of a radioimmunoassay for histamine measurement in biological fluids. J. Allergy Clin. Immunol. 82,638-646.

McCAY, P.B., PFEIFER, P.M. & STIPE, W.H. (1972). Vitamin E protection of membrane lipids during electron transport functions. Ann. N.Y. Acad. Sci., 203, 62-73.

McEACHERN, A.E., SHELTON, E.R., BHAKTA, S., OBERNOLTE, R., BACH, C., ZUPPAN, P., FIJISAKA, J., ALDRICH, R.W. & JARAGIN, K. (1991). Expression cloning of a rat B_2 receptor. Proc. Natl Acad. Sci. U.S.A. 88, 7724-7728.

MIADONNA, A., TEDESCHI, A., LEGGIERI, E., LORNI, M., FOLCO, G., SALA, A., QUALIZZA, R., FROLDI, M. & ZANUSSI, C. (1987). Behaviour and clinical relevance of histamine and leukotrienes C_4 and B_4 in grass pollen-induced rhinitis. Am. Rev. Respir. Dis. 136, 357-362.

MIADONNA, A., TEDESCHI, A., ARNOUX, B., SALA, A., ZANUSSI, C. & BENVENISTE, J. (1989). Evidence of PAF-acether metabolic pathway activation in antigen challenge of upper respiratory airways. Am. Rev. Respir. Dis. 140, 142-147.

MISAWA, M. (1988). A new rhinitis model using chemical mediators in rats. Japan. J. Pharmacol. 48, 15-22.

MISAWA, M. & IWAMURA, S. (1990). Platelet-activating factor (PAF-acether)-induced rhinitis and involvement of PAF in allergic rhinitis in guinea pigs. Japan J. Pharmacol. 54, 217-226.

MIZRAHI, J., D'ORLEANS-JUSTE, P., CARANIKAS, S. & REGOLI, D. (1982). Effects of peptides and amines on isolated guinea pig trachea as influenced by inhibitors of the metabolism od arachidonic acid. Pharmacology 25, 320-326.

MIZUNO, H., KAWAMURA, Y., IWASE, N. & OHNO, H. (1990). Effects of flutropium on experimental models of drug and allergen-induced rhinitis in guinea-pigs. Japan J. Pharmacol. 55, 321-328.

MONTEFORT, S., FEATHER, I.H., WILSON, S.J., HASKARD, D.O., LEE, T.H., HOLGATE, S.T. & HOWARTH, P.H. (1992). The expression of leukocyte-endothelial adhesion molecules is increased in perennial allergic rhinitis. Am. J. Respir. Cell Molec. Biol. 7, 393-398.

MOREL, A.M. & DELAAGE, M.A. (1988). Immunoanalysis of histamine through a novel chemical derivatization. J. Allergy Clin. Immunol. 82, 646-654.

MORLEY, J., PAGE, C.P. & SANJAR, S. (1985). PAF and airway hyperreactivity. Lancet 2,451.

MOUSLI, M., BRONNER, C., BUEB, J.L., TSCHIRHART, E., GIES, J.P. & LANDRY, Y. (1989). Activation of rat peritoneal mast cells by substance P and mastoparan. J. Pharmacol. Exp. Ther. 250, 329-335.

MURLAS, C.G. & ROUM, J.H. (1985). Sequence of pathologic changes in the airway mucosa of guinea pigs during ozone-induced bronchial hyperreactivity. Am. Rev. Respir. Dis., 131, 314-320.

MYGIND, N. & ANGGARD, A. (1984). Anatomy and physiology of the nose - pathophysiological alterations in allergic rhinitis. Clin. Rev. Allergy 2, 173-188.

NACLERIO, R.M., MEIER, H.L. & ADKINSON, N.F. (1983a). *In vivo* demonstration of inflammatory mediator release following nasal challenge with antigen. Eur. J. Respir. Dis. 128, 26-32.

NACLERIO, R.M., MEIER, H.L., KAGEY-SOBOTKA, A., ADKINSON, N.F., MEYERS, D.A., NORMAN, P.S. & LICHTENSTEIN, L.M. (1983b). Mediator release after nasal airway challenge with allergen. Am. Rev. Respir. Dis., 128, 597-602.

NACLERIO, R.M., PROUD, D., TOGIAS, A., KAGEY-SOBOTKA, A., ADKINSON, N.F., MEYERS, D.A., PLAVT, M., NORMAN, P.S. & LICHTENSTEIN, L.M. (1985). Inflammatory mediators in late antigen-induced rhinitis. New Eng. J. Med. 313, 65-70.

NACLERIO, R.M., PROUD, D., LICHTENSTEIN, L.M., KAGEY-SOBOTKA, A., HENDLEY, S., SORRENTINO, J.V. & GWALTNEY, J.M. (1987). Kinins are generated during experimental rhinovirus colds. J. Infec. Dis. 157,133-141.

NACLERIO, R.M., PROUD, D., KAGEY-SOBOTKA, A., FREIDHOFF, L., NORMAN, P.S. & LICHTENSTEIN, L.M. (1989). The effect of cetirizine on early allergic response. Laryngoscope 99, 596-599.

NACLERIO, R.M., KAGEY-SOBOTKA, A., LICHTENSTEIN, L.M., FREIDHOFF, L. & PROUD, D. (1990). Terfenadine, an H_1 antihistamine, inhibits histamine release *in vivo* in man. Am. Rev. Respir. Dis. 142, 167-171.

NACLERIO, R.M. & TOGIAS, A.G. (1991). The nasal allergic reaction: observations on the role of histamine. Clin. Exp. Allergy 21, 13-19.

NEWBALL, H.H., TALAMO, R.C. & LICHTENSTEIN, L.M. (1979). Anaphylactic release of a basophil kallikrein-like activity. Purification and characterization. J. Clin. Invest. 64, 457-465.

NOLTE, D. & ULMER, W.T. (1966). Measurement of nasal resistance with the whole body plethysmograph. Med. Thor. 23, 349-357.

NOLTE, D. & LUDER-LUHR, I. (1973). Comparing measurements of nasal resistance by body plethysmography and by rhinomanometry. Respiration 30, 31-38.

O'BYRNE, P.M. & FULLER, R.W. (1989). The role of thromboxane A_2 in the pathogenesis of airway hyperresponsiveness. Eur. Respir. J. 2, 782-786.

O'CONNER, B.J., EVANS, P.M. RIDGE, S.M., FULLER, R.W. & BARNES, P.J. (1991). Effect of an inhaled steroid (budesonide) on indirect airway responsiveness and eosinophils in asthma. Am. Rev. Respir. Dis. 143, A422.

O'FLAHERTY, J.T. & WYLKLE, R.L. (1983). Biology and biochemistry of platelet activating factor. Clin. Rev. Allergy, 1, 353-367.

OHNISHI, M., RUHNO, J., DOLOVICH, J. & DENBURG, J.A. (1988). Allergic rhinitis and mucosal conditioned medium stimulates growth and differentiation of basophil/mast cell and eosinophil progenitors from atopic blood. J. Allergy Clin. Immunol. 81, 1149-1154.

OKUDA, M., WATASE, T., MAZAWA, A. & LIU, C.M. (1988). The role of leukotriene D_4 in allergic rhinitis. Ann. Allergy 60,537-40.

PAGE, C.P. (1988). The role of platelet-activating factor in asthma. J. Allergy Clin. Immunol. 81, 144-150.

PAGE, C.P. & COYLE, A.J. (1990). Late phase allergic reactions. In: Asthma, Dorsch, W. ed. CRC Press. p.72-81.

PAGE, C.P. (1991). One explanation for the asthma paradox: inhibition of natural anti-inflammatory mechanism by β_2-agonists. Lancet 337, 982.

PALMBLAD, J., MALMSTEN, C.L., UDEN, A.M., RADMARK, O., ENGSTEDT, L. & SAMUELSSON, B. (1981). Leukotriene B_4 is a potent and stereospecific stimulator of neutrophil chemotaxis and adherence. Blood 58, 658-661.

PATTERSON, R., BERNSTEIN, P.R., HARRIS, K.E. & KRELL, R.D. (1984). Airway responses to sequential challenge with platelet-activating factor and leukotriene D_4 in rhesus monkeys. J. Lab. Clin. Med., 104, 340-345.

PERKINS, M.N., BURGESS, G.M., CAMPBELL, E.A., HALLETT, A., MURPHY, R.J., NAEEM, S., PATEL, I.A., PATEL, S., RUEFF, A. & DRAY, A. (1991). HOE 140: a novel bradykinin analogue that is a potent antagonist at both B_2 and B_3 receptors *in vitro*. Br. J. Pharmacol. 102, 171P.

PEYRET, L.M., MOREAU, P., DOLLUC, J. & GEFFARD, M. (1986). Antibodies to histamine. J. Immunol. Methods 90, 39-45.

PINCUS, S.H. (1983). Hydrogen peroxide release from eosinophils: quantitative, comparative studies of human and guinea-pig eosinophils. J. Invest. Dermatol. 80, 278-281.

PINCKARD, R.N., JACKSON, E.M., HOPPENS, C., WEINTRAUB, S.T., LUDWIG, J.C., McMANUS, L.M. & MOTT, G.E. (1984). Molecular heterogeneity of platelet-activating factor produced by stimulated human polymorphonuclear leukocytes. Biochem. Biophys. Res. Commun. 122, 325-332.

PIOTROWSKI, W. & FOREMAN, J.C. (1986). Some effects of calcitonin gene-related peptide in human skin and on histamine release. Br. J. Dermatol. 114, 37-46.

PIPKORN, U., KARLSSON, G. & BAKE, B. (1984). Effect of platelet activating factor on the human nasal mucosa. Allergy 39, 141-145.

PIPKORN, U., PROUD, D., SCHLEIMER, R.P., PETERS, S.P., ADKINSON, N.F., KAGEY-SOBOTKA, A., NORMAN, P.S., LICHTENSTEIN, L.M. & NACLERIO, R.M. (1987a). Effects of short-term systemic glucocorticoid treatment on human nasal mediator release after antigen challenge. J. Clin. Invest. 80, 957-961.

PIPKORN, U., PROUD, D., LICHTENSTEIN, L.M., KAGEY-SOBOTKA, A., NORMAN, P.S. & NACLERIO, R.M. (1987b). Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. N. Eng. J. Med. 316, 1506-1510.

PIPKORN, U., KARLSSON, G. & ENERBACH, L. (1988). Cellular response of the allergic human nasal mucosa to natural allergen exposure. J. Allergy Clin. Immunol. 82, 1046-1054.

PIPKORN, U. (1989). Mediators and nasal allergy. Clin. Exp. Allergy 19,585-589.

PIPKORN , U. (1990). H_1-antagonists as a tool in clarifying the pathophysiology of nasal allergy. Clin. Exp. Allergy 20, 25-29.

PLAUT, M., PIERCE, J.H., WATSON, C.J., HANLEY-HYDE, J., NORDAN, R.P. & PAUL, W.E. (1989). Mast cell lines produce lymphokines in response to cross-linkage of FcϵRI or to calcium inophores. Nature 339, 64-67.

PLEVIN, R. & OWEN, J. (1988). Multiple B_2 kinin receptors in mammalian tissues. TIPS 9, 387-389.

POLOSA, R. (1993). Role of kinin-kallikrein pathway in allergic diseases. Allergy 48, 217-225.

PONGRACIC, J.A., NACLERIO, R.M., REYNOLDS, C.J. & PROUD, D. (1991). A competitive kinin receptor antagonist, [D-Arg^0,Hyp^3,D-Phe^7]-bradykinin, does not affect the response to nasal provocation with bradykinin. Br. J. Clin. Pharmacol. 31, 287-294.

PROUD, D., TOGIAS, A., NACLERIO, R.M., CRUSH, S.A., NORMAN, P.S. & LICHTENSTEIN, L.M. (1983). Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. J. Clin. Invest. 72, 1678-85.

PROUD, D., MACGLASHAN, D.W., NEWBALL, H.H., SHULMAN, E.S. & LICHTENSTEIN, L.M. (1985). Immunoglobulin E-mediated release of a kininogenase from purified human lung mast cells. Am. Rev. Respir. Dis. 132, 405-408.

PROUD, D., BAUMGARTEN, C.R., NACLERIO, R.M. & WARD, P.E. (1987). Kinin metabolism in human nasal secretions during experimentally induced allergic rhinitis. J. Immunol. 138, 428-434.

PROUD, D., REYNOLDS, C.J., LACAPRA, S., KAGEY-SOBOTKA, A., LICHTENSTEIN, L.M. & NACLERIO, R.M. (1988). Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. Am. Rev. Respir. Dis. 137, 613-6.

PROUD, D., NACLERIO, R.M., GWALTNEY, J.M. & HENDLEY, J.O. (1990). Kinins are generated in nasal secretions during natural rhinovirus colds. J. Infect. Dis. 161, 120-123.

RAJAKULASINGAM, K., POLOSA, R., HOLGATE, S.T. & HOWARTH, P.H. (1991). Comparative effects of bradykinin, kallidin and [Des-Arg^9]-bradykinin in atopic rhinitic and normal volunteers. J. Physiol. 437, 557-87.

RAJAKULASINGAM, K., POLOSA, R., LAU, L.C.K., CHURCH, M.K., HOLGATE, S.T. & HOWARTH, P.H. (1992a). The influence of terfenadine and ipratropium bromide alone and in combination on bradykinin-induced nasal symptoms and plasma protein leakage. Clin. Exp. Allergy 22, 717-723.

RAJAKULASINGAM, K., POLOSA, R., LAU, L.C.K., CHURCH, M.K., HOLGATE, S.T. & HOWARTH, P.H. (1992b). Nasal effects of bradykinin and capsaicin: influence on plasma protein leakage and role of sensory neurons. J. Appl. Physiol. 72, 1418-1424.

RAJAKULASINGAM, K., POLOSA, R., LAU, L.C.K., CHURCH, M.K., HOLGATE, S.T. & HOWARTH, P.H. (1993). Comparative nasal effects of bradykinin and histamine: influence on nasal airways resistance and plasma protein exudation. Thorax 48, 324-329.

RAPHAEL, G.D., DRUCE, H.M., BARANUIK, J.N. & KALINER, M.A. (1988). Pathophysiology of rhinitis: assessment of the sources of protein in methacholine-induced nasal secretions. Am. Rev. Respir. Dis. 138, 413-420.

RAPHAEL, G.D., BARANIUK, J.N. & KALINER, M.A. (1991). How and why the nose runs. J. Allergy Clin. Immunol. 87, 457-467.

RAWSON, R.A. (1943). The binding of T-1824 and structurally related diazo dyes by the plasma proteins. Am. J. Physiol. 138, 708-717.

REGOLI, D., BARABE, J. & PARK, W.K. (1977). Receptors for bradykinin in rabbit aortae.

Can. J. Physiol. Pharmacol. 55, 855-867.

REGOLI, D., MARCEAU, F. & BARABE, J. (1978). *De novo* formation of vascular receptor for bradykinin. Can. J. Physiol. Pharmacol. 56, 674-677.

REGOLI, D. & BARABE, J. (1980). Pharmacology of bradykinin and related kinins. Pharmacol. Rev. 32, 1-46.

REGOLI, D., MARCEAU, J. & LAVIGNE, J. (1981). Induction of B_1 receptors for kinins in the rabbit by a bacterial lipopolysaccharide. Eur J. Pharmacol. 71, 105-115.

REGOLI, D., DRAPEAU, G., ROVEN, P., DION, S., D'ORLEANS-JUSTE, P. & BARABE, J. (1986). The actions of kinin antagonists on B_1 and B_2 receptor systems. Eur. J. Pharmacol. 123, 61-65.

REGOLI, D., RHALEB, N., DION, S. & DRAPEAU, G. (1990). New selective bradykinin receptor antagonists and bradykinin B_2 receptor characterisation. TIPS 11, 156-61.

RHALEB. N.E., DION, S., D'ORLEANS-JUSTE, P., DRAPEAU, G., REGOLI, D. & BROWNE, R.G. (1988). Bradykinin antagonism: differentiation between peptide antagonists and anti-inflammatory agents. Eur. J. Pharmacol. 151, 275-279.

RHALEB, N.E., GOBEIL, F. & REGOLI, D. (1992). Non-selectivity of new bradykinin antagonists for B_1 receptors. Life Sci. 51, 125-129.

RICHERSON, H.B. & SEEBOHM, P.M. (1968). Nasal airway response to exercise. J. Allergy 41, 269-284.

RIFO, J., POURRAT, M., VAVREK, R.J., STEWART, J.M. & HUIDOBRO-TORO, J.P. (1987). Bradykinin receptor antagonists used to characterise the heterogeneity of bradykinin-induced responses in rat vas deferens. Eur. J. Pharmacol. 142, 305-312.

ROBERTSON, D.N., COYLE, A.J., RHODEN, K.J., GRANDORDY, B., PAGE, C.P. & BARNES, P.J. (1988). The effect of platelet-activating factor on histamine and muscarinic receptor function in guinea-pig airways. Am. Rev. Respir. Dis. 137, 1317-1322.

ROGERS, D.F., BOSCHETTO, P. & BARNES, P.J. (1989). Plasma exudation. Correlation between Evans blue dye and radiolabelled albumin in guinea-pig airways *in vivo*. J. Pharmacol. Methods 21, 309-315.

ROGERS, D.F., DIJK, S. & BARNES, P.J. (1990). Bradykinin-induced plasma exudation in guinea-pig airways: Involvement of platelet-activating factor. Br. J. Pharmacol. 101, 739-745.

ROKENES, H.K., ANDERSSON, B. & RUNDCRANTZ, H. (1988). Effect of terfenadine and placebo on symptoms after nasal allergen provocation. Clin. Allergy 18, 63-69.

ROTHLEIN, R., CZAIJKOWSKI, M., O'NEILL, M.M., MARLIN, S.D., MAINOLFI, E. & MERLUZZI, V.J. (1988). Induction of intercellular adhesion molecule-1 on primary and continuous cell lines by proinflammatory cytokines. J. Immunol. 141, 1665-1669.

RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate

endothelium-derived relaxing factor. Am. J. Physiol., 250, H822-827.

RUNDCRANTZ, H. (1964). Posture and congestion of nasal mucosa in allergic rhinitis. Acta. Otolaryngol. 58, 283-287.

SAID, S.I. & MUTT, V. (1988). Vasoactive intestinal peptide and related peptides. Ann. N.Y. Acad. Sci. 527,1-688.

SAKAMOTO, T., TSUKAGOSHI, H., BARNES, P.J. & CHUNG, K.F. (1993). Role played by NK_2 receptor and cyclooxygenase activation in bradykinin B_2 receptor mediated-airway effects in guinea-pigs. Agents Actions 39, 111-117.

SALMAN, S.D., PROCTOR, D.F., SWIFT, D.L. & EVERING, S.A. (1971). Nasal resistance: Description of a method and effect of temperature and humidity. Ann. Otol. Rhinol. Laryngol. 80, 736-743.

SANDFORD, A.J., SHIRAKAWA, T., MOFFAT, M.F., DANIELS, S.E., RA, C., FAUX, J.A., YOUNG, R.P., NAKAMURA, Y., LATHROP, G.M., COOKSON, W.O.C.M. & HOPKIN, J.M. (1993). Localisation of atopy and β subunit of high-affinity IgE receptor (FcϵRI) on chromosome 11q. Lancet 341, 332-334.

SANJAR, J.L., AOKI, S., BOUBEKEUR, K., CHAPMAN, I.D., SMITH, D., KINGS, M.A. & MORLEY, J. (1990). Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet-activating factor: effect of anti-asthma drugs. Br. J. Pharmacol., 99, 267-272.

SARIA, A., MARTLING, C.R., YAN, Z., THEODORRSON, N.E., GAMSE, R., LUNDBERG, J.M. (1988). Release of multiple tachykinins from capsaicin-sensitive sensory neurones in the lung by bradykinin, histamine, dimethyl piperazinium and vagal nerve stimulation. Am. Rev. Respir. Dis. 137, 1330-1335.

SASAKI, M., HERD, C.M. & PAGE, C.P. (1993). Effect of heparin and a low-molecular weight heparinoid on PAF-induced airway responses in neonatally immunized rabbits. Br. J. Pharmacol. 110,107-112.

SCHACHTER, M., UCHIDA, Y., LONGRIDGE, D.J., LABEDZ, T., WALLEY, E.T., VAVREK, R. & STEWART, J.M. (1987). New synthetic antagonists of bradykinin. Br. J. Pharmacol. 92, 851-855.

SEGUIN, L., WIDDOWSON, P.S., GIESEN-CROUSE, E. (1992). Existence of three subtypes of bradykinin B_2 receptors in guinea pig. J. Neurochem., 59, 2125-2133.

SELTZER, J., BIGBY, B.G., STULBARG, M., HOLTZMAN, M.J., NADEL, J.A., UEKI, I.F., LEIKAUF, G.D. & GOETZL, E.J. (1986). O_3^- induced change in bronchial reactivity to methacholine and airway inflammation in humans. J. Appl. Physiol., 60, 1321-1326.

SHAW, R.J., FITZHARRIS, P., CROMWELL, O., WARDLAW, A.J. & KAY, A.B. (1985). Allergen-induced release of sulphidopeptide leukotrienes (SRS-A) and LTB_4 in allergic rhinitis. Allergy 40, 1-6.

SHAW, R.J., WALSH, G.M., CROMWELL, O., MOQBEL, R., SPRY, G.J.F. & KAY, A.B.

(1985). Activated human eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation. Nature 316, 150-152.

SHEIKH, I.A. & KAPLAN, A.P. (1986). Studies of the digestion of bradykinin, lysyl-bradykinin and [Des-Arg^9]-bradykinin by angiotensin converting enzyme. Biochem. Pharmacol. 35, 1951-1956.

SHEN, T.Y., HWANG, S.B., CHANG, M.N., DOEBBER, T.W., LAM, M.H., WU, M.S., WANG, X., HAN, G.Q. & LI, R.Z. (1985). Characterisation of a platelet-activating factor receptor antagonist isolated from haifenteng (*Piper futokadsura*): specific inhibition of *in vitro* and *in vivo* platelet-activating factor-induced effects. Proc. Natl Acad. Sci. U.S.A. 82, 672-676.

SHORE, P.A., BURKHALTER, A. & COHN, V.H. (1959). A method for the fluorimetric assay of histamine in tissues. J. Pharmacol. Exp. Ther. 127, 182-186.

SHUKLA, S.D. (1992). Platelet-activating factor receptors and signal transduction mechanisms. FASEB J. 6, 2296-2301.

SHULT, P.A., GRAZIANO, F.M. & BUSSE, W.W. (1986). Enhanced eosinophil luminol-dependent chemiluminescence in allergic rhinitis. J. Allergy Clin. Immunol. 77, 702-708.

SIBBALD, B. & RINK, E. (1991). Epidemiology of seasonal and perennial rhinitis; clinical presentation and medical history. Thorax 46, 895-901.

SIMONS, F.E.R. & SIMONS, K.J. (1988). H_1 receptor antagonist treatment of chronic rhinitis. J. Allergy Clin. Immunol. 81, 975-980.

SNYDER, F. (1985). Chemical and biochemical aspects of platelet activating factor: A novel class of acetylated ether-linked choline phospholipids. Med. Res. Rev. 5, 107-140.

SNYDER, F. (1990). Platelet-activating factor and related acetylated lipids as potent biologically active cellular mediators. Am. J. Physiol. 259, C697-C708.

SNYDER, S.H. & SNOWMAN, A.M. (1987). Receptor effects of cetirizine. Ann. Allergy 59, 4-8.

SOLER, M., SIELCZAK, M. & ABRAHAM, W.M. (1990). A bradykinin-antagonist blocks antigen-induced airway hyperresponsiveness and inflammation in sheep. Pulmonary Pharmacol. 3, 9-15.

SONDHI, M.M. & GOPINATH, B. (1971). Determination of vocal-tract shape from impulse response at the lips. J. Acoust. Soc. Am. 49, 1867-1873.

SPINA, D., McKENNIFF, M.G., COYLE, A.J., SEEDS, E.A.M., TRAMONTANA, M., PERRETTI, F., MANZINI, S. & PAGE, C.P. (1991). Effect of capsaicin on PAF-induced bronchial hyperresponsiveness and pulmonary cell accumulation in the rabbit. Br. J. Pharmacol. 103, 1268-1274.

SPRY, C.J.F. (1985). Synthesis and secretion of eosinophil granule substances. Immunol. Today 6, 332-335.

SPRAGG, J., VAVREK, R.J. & STEWART, J.M. (1988). The inhibition of glandular kallikrein by peptide analog antagonists of bradykinin. Peptides 9, 203-206.

STERANKA, L.R., FARMER, S.G. & BURCH, R.M. (1989). Antagonists of B_2 bradykinin receptors. FASEB J. 3, 2019-2025.

STOKSTED, P. (1952). The physiologic cycle of the nose under normal and pathological conditions. Acta Otolaryngol. 42, 175-179.

SUNDAY, M.E., KAPLAN, L.M., MOTOYAMA, E., CHIN, W.W. & SPINDEL, E.R. (1988). Gastrin releasing peptide (mammalian bombesin) gene expression in health and disease. Lab. Invest. 59, 5-24.

SVENSSON, C., ANDERSSON, M., PERSSON, C.G.A., VENGE, P., ALKNER, U. & PIPKORN, U. (1990). Albumin, bradykinin and eosinophil cationic protein on the nasal mucosal surface in patients with hayfever during natural allergen exposure. J. Allergy Clin. Immunol. 85, 828-33.

SZAREK, J.L. & SCHMIDT, N. (1990). Hydrogen peroxide-induced potentiation of contractile responses in isolated rat airways. Am. J. Physiol., 258, L232-237.

SZCZEKILK, A. (1987). Antioxidant effect of vitamin E in hyperlipoproteinemias. Agents & Actions, 22, 359.

TAYLOR, S.C., LAMDEN, M.P. & TAPPEL, A.L. (1976). Sensitive fluorimetric method for tissue tocopherol analysis. Lipids, 11, 530-538.

TAYLOR, G.W., TAYLOR, I., BLACK, P., MALTBY, N.H., TURNER, N., FULLER, R.W. & DOLLERY, C.T. (1989). Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. Lancet 1,585-8.

TERASHITA, Z., TSUSHIMA, S., YOSHIOKA, Y., NOMURA, H., INADA, Y. & NISHIKAWA, K. (1983). CV-3988, a specific antagonist of platelet activating factor (PAF). Life Sci. 32,1975-1982.

TOGO, J., BURCH, R.M., DEHAAS, C.J., CONNOR, J.R. & STERANKA, L.R. (1989) D-Phe^7-substituted peptide bradykinin antagonists are not substrates for kininase II. Peptides 10, 109-112.

TOWNLEY, R.G., RYO, U.Y., KOLOTKIN, B.M. & KAY, B. (1975). Bronchial sensitivity to methacholine in current and former asthmatic and rhinitic patients and control subjects. J. Allergy Clin. Immunol. 56, 429-442.

TRIFILIEFF, A., HADDAD, E., LANDRY, Y. & GIES, J.P. (1991). Evidence for two high-affinity bradykinin binding sites in guinea-pig lung. Eur. J. Pharmacol. 207, 129-134.

TRIFILIEFF, A., DA SILVA, A., LANDRY, Y. & GIES, J.P. (1992). Effect of Hoe 140, a new B_2 noncompetitive antagonist on guinea pig tracheal bradykinin receptors. J. Pharmacol. Exp. Ther. 263, 1377-1382.

UDAKA, K., TATENCHI, Y. & MOVAT, H.Z. (1970). Simple method for quantitation of

enhanced vascular permeability. Proc. Soc. Exp. Biol. Med. 133, 1384-1387.

VARGAFTIG, B.B., LEFORT, J., CHIGNARD, M. & BENVENISTE, J. (1980). Platelet-activating factor induces a platelet dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. Eur. J. Pharmacol. 65, 185-192.

VAVREK, R.J. & STEWART, J.H. (1985). Competitive antagonists of bradykinin. Peptides 6, 161-164.

VENGE, P., ROXIN, L.E. & OLSSON, I. (1977). Radioimmunoassay of human eosinophil cationic protein. Br. J. Haematol. 37, 331-335.

VIEGAS, M., GOMEZ, E., BROOKS, J., GATLAND, D. & DAVIES, R.J. (1987). Effect of the pollen season on nasal mast cells. Br. Med. J. 294, 414.

WARDLAW, A.J., MOQBEL, R., CROMWELL, O. & KAY, A.B. (1986). Platelet-activating factor. A potent chemotactic and chemokinetic factor from human eosinophils. J. Clin. Invest. 78, 1701-1706.

WARDLAW, A.J., CHUNG, K.F., MOQBEL, R., MACDONALD, A.J., HARTNELL, A., McCLUSTER, M., COLLINS, J.V., BARNES, P.J. & KAY, A.B. (1990). Effects of inhaled PAF in man in circulating and bronchoalveolar lavage neutrophils: relationship to bronchoconstriction and changes in airway responsiveness. Am. Rev. Respir. Dis. 141, 386-392.

WEBBER, S.E., MORIKAWA, T. & WIDDICOMBE, J.G. (1992). PAF-induced muscarinic cholinoceptor hyperresponsiveness of ferret tracheal smooth muscle and gland secretion *in vitro*. Br. J. Pharmacol., 105, 230-237.

WISEMAN, J., WHEELDON, A. & BIRCH, P.J. (1994). Characterisation of receptors mediating bradykinin and [Des-Arg^9]-bradykinin-induced paw oedema in the mouse. Br. J. Pharmacol. In press.

WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALPERMANN, H.G., ANAGNOSTOPOULOS, H., HENKE, S., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHOLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: *in vivo* studies. Br. J. Pharmacol. 102, 774-777.

WOOLCOCK, A.J., SALOME, C.M. & YAN, K. (1984). The shape of the dose-response curve to histamine in asthmatic and normal subjects. Am. Rev. Respir. Dis. 130, 71-75.

YEADON, M., WILKINSON, D. & PAYNE, A.N. (1990). Ozone induces bronchial hyperreactivity to inhaled substance P by functional inhibition of enkephalinase. Br. J. Pharmacol. 99, 191P.

ZWAARDEMAKER, H. (1891). Athembeschlag als Hulfsmittel zur Diagnose der nasalen Stenose. Arch. Laryngol. Rhinol. 1, 174-177.

Responses to comments by the examiners

Page 2: Line 7. Insert "In addition to the human studies, an attempt was made to develop a guinea pig model for quantifying vascular permeability of the nasal mucosa, with the intention for future use of this model for investigation of kinins and other inflammatory mediators in the nasal airway.

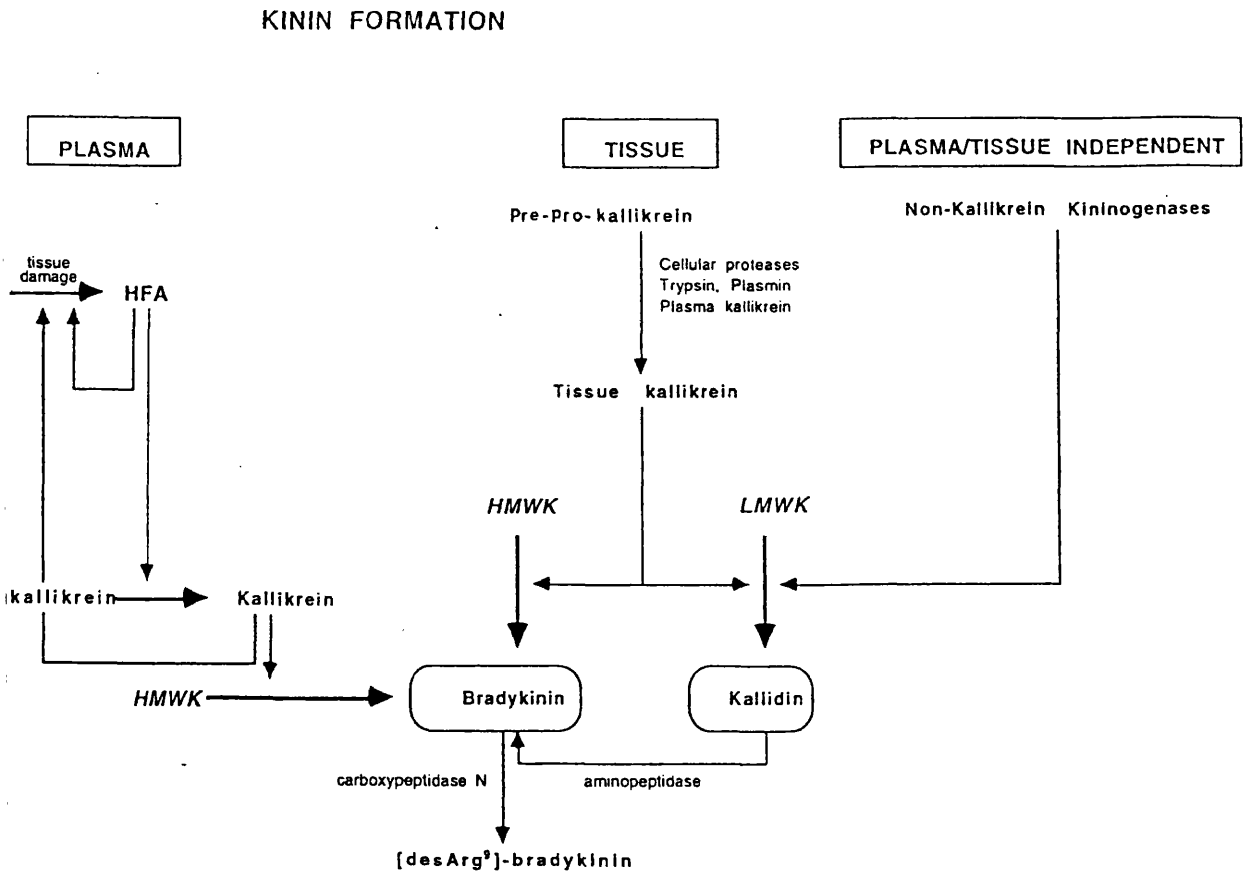
Page 18: Line 15. Insert "to" between `according' and `the'.

Page 19: However other studies subsequent to the work of Cookson et al., (1989) which have further assessed the linkage between chromosome 11q and atopy have been unable to confirm a significant link between the chromosome 11q13 marker D11S97 and atopy, as defined by either raised IgE or by positive skin prick testing (Lympany *et al.*, 1992a). Lympany *et al.*, (1992b) have extended their studies to investigating linkage between this chromosomal region and bronchial hyperresponsiveness, characteristic of asthma, but again were unable to show a significant correlation between this region and bronchial hyperresponsiveness to methacholine. Other workers have also rejected a linkage between atopy and the D11S97 marker on human chromosome 11q (Rich *et al.*, 1992; Hizawa *et al.*, 1992). Hizawa *et al.*, (1992) suggest a possible heterogeneity in the genetic elements of atopy and at this stage, a genetic predisposition for the development of atopy cannot be excluded, although the current reports on this should perhaps be approached with caution.

Page 22: Line 13. Insert "However, whether the ability of PAF to induce bronchial hyperresponsiveness differs between asthmatic and normal subjects is a point of controversy, since several studies in non-asthmatic subjects have failed to demonstrate such a effect of PAF (Lai *et al.*, 1990; Spencer *et al.*, 1990; Gebremichael & Leuenberger, 1992).

Page 22: Line 27. Insert "The ability of adhesion molecules to induce such nasal inflammation may be further enhanced by a positive feedback mechanism, in which cytokines upregulate ICAM-1 on human nasal epithelial cells (Altman *et al.*, 1993). Various studies have shown that expression of the adhesion molecules, ELAM-1 and ICAM-1, can be induced by the cytokines, IL-1, TNF-α (Groves *et al.*, 1991), IL-6 (Bajaj *et al.*, 1993), and interferon-γ (Das *et al.*, 1993).

Page 35: Line 15. Insert diagram.



"Diagram showing mechanisms of kinin formation (Taken from, Polosa, 1993).
HF=Hageman factor, factor XII
HFA=activated Hageman factor
HMWK=high molecular weight kininogen
LMWK=low molecular weight kininogen".

Page 43: Line 25. Insert "non peptide" between `specific' and `receptor'.

Page 49: Line 22. Insert "at 21^{0}C" between `temperature' and `and'.

Page 55: Line 2. Insert after `....less than 20%.' "The baseline measurements taken by acoustic rhinometry and posterior rhinomanometry were such that a normal distribution was assumed, therefore.....".

Page 61: Line 4. Insert "at" between `assayed' and `the'.

Page 62: Line 9. Insert " A fluorimetric histamine assay was used to measure histamine release from rat peritoneal mast cells, rather than the radioimmunoassay for histamine previously described (section 2.3.2). This is considerably less expensive and is appropriate in these samples since there was minimal protein content, in contrast to the nasal lavage samples. Therefore, interference from protein in the assay was not a concern and this method is generally adopted in the published literature.

Page 62: Line 9 after Shore et al., (1959) Insert " and is sensitive to approximately 2ng/ml.

Page 94: Line 2. Delete "bradykininis". Insert "bradykinin is".

Page 131: At end of legend, insert "Cetirizine or BKA2 alone, had no effect on nasal airway resistance".

Page 141: Line 11. Delete "resiues". Insert " residues".

Page 147: Line 6. Insert after... (Figure 7.1). "Point by point comparison is shown rather than area under the curves since both resulted in the same answer".

Page 171: Line 2. Delete "1nM". Insert "10nM".

Page 172: Line 3. Insert "Unfortunately, limitations in terms of time did not allow for further modification to be undertaken. However,.....".

Page 175: Line 9. Delete "inflammed". Insert "inflamed".

Page 175: Line 28. Delete "hypersensitivty". Insert "hypersensitivity".

e 174: Line 4. Insert diagram.

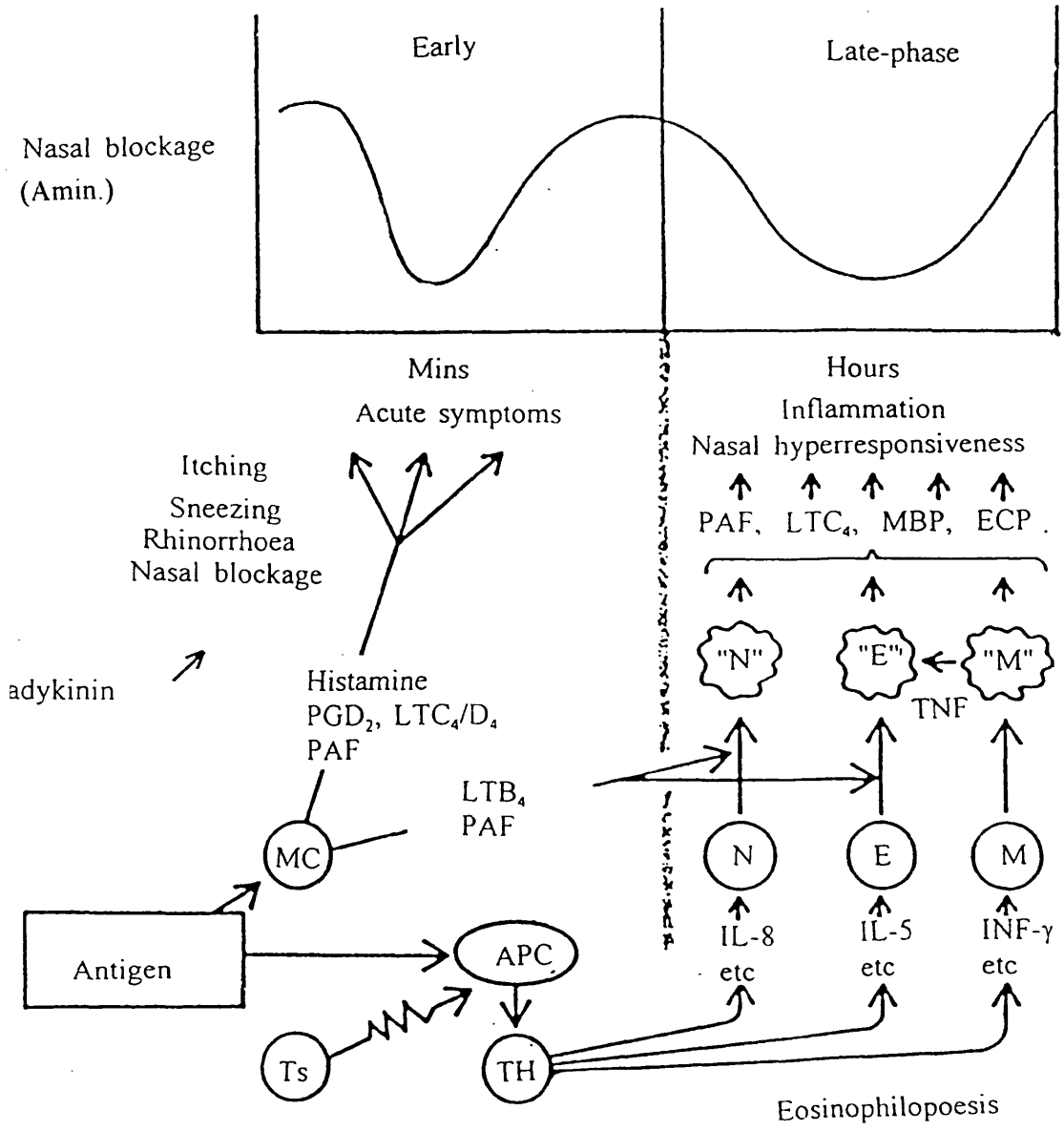


Diagram illustrating the pathophysiology of allergic rhinitis.

174: Line 5. Insert diagram.

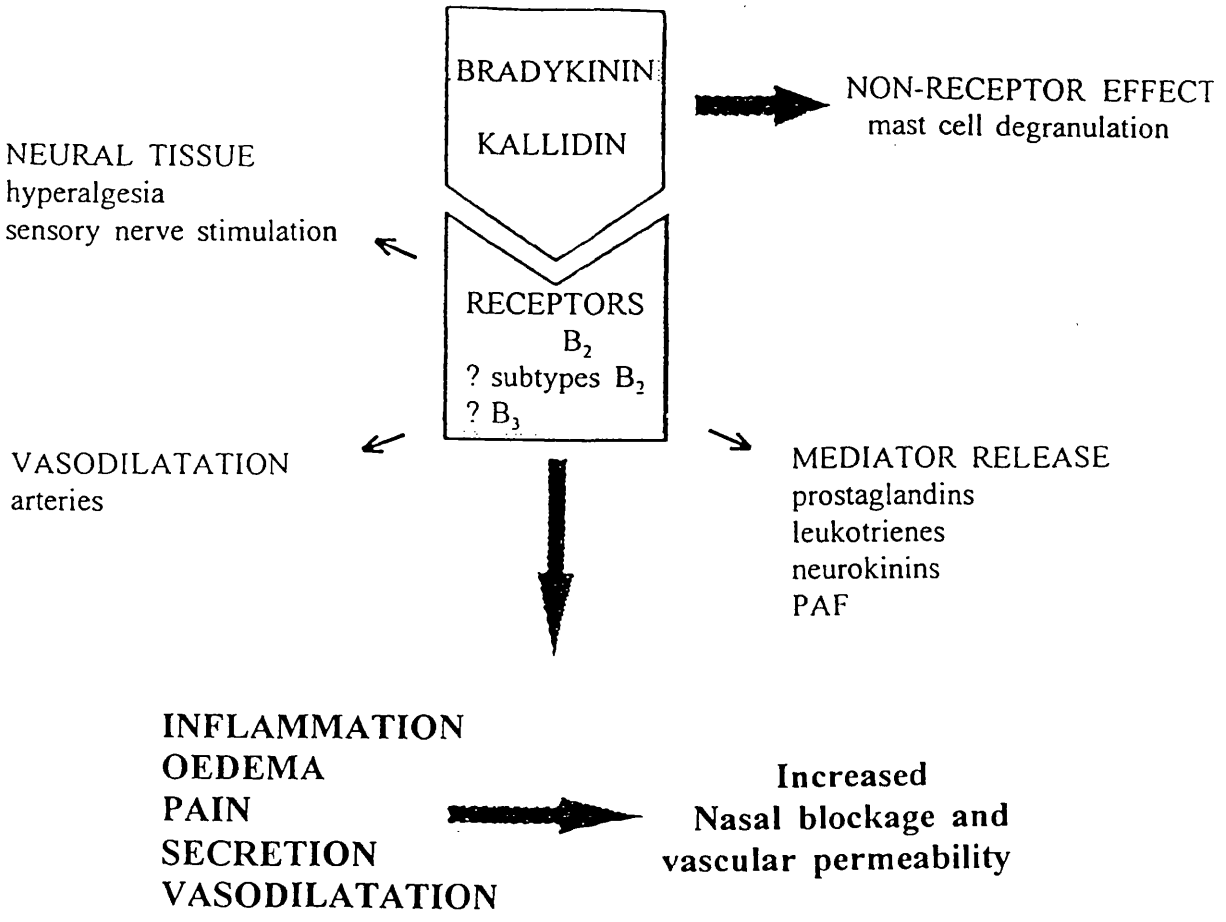


Diagram summarising nasal effects of kinins.

ıge 175: Line 16. Insert diagram.

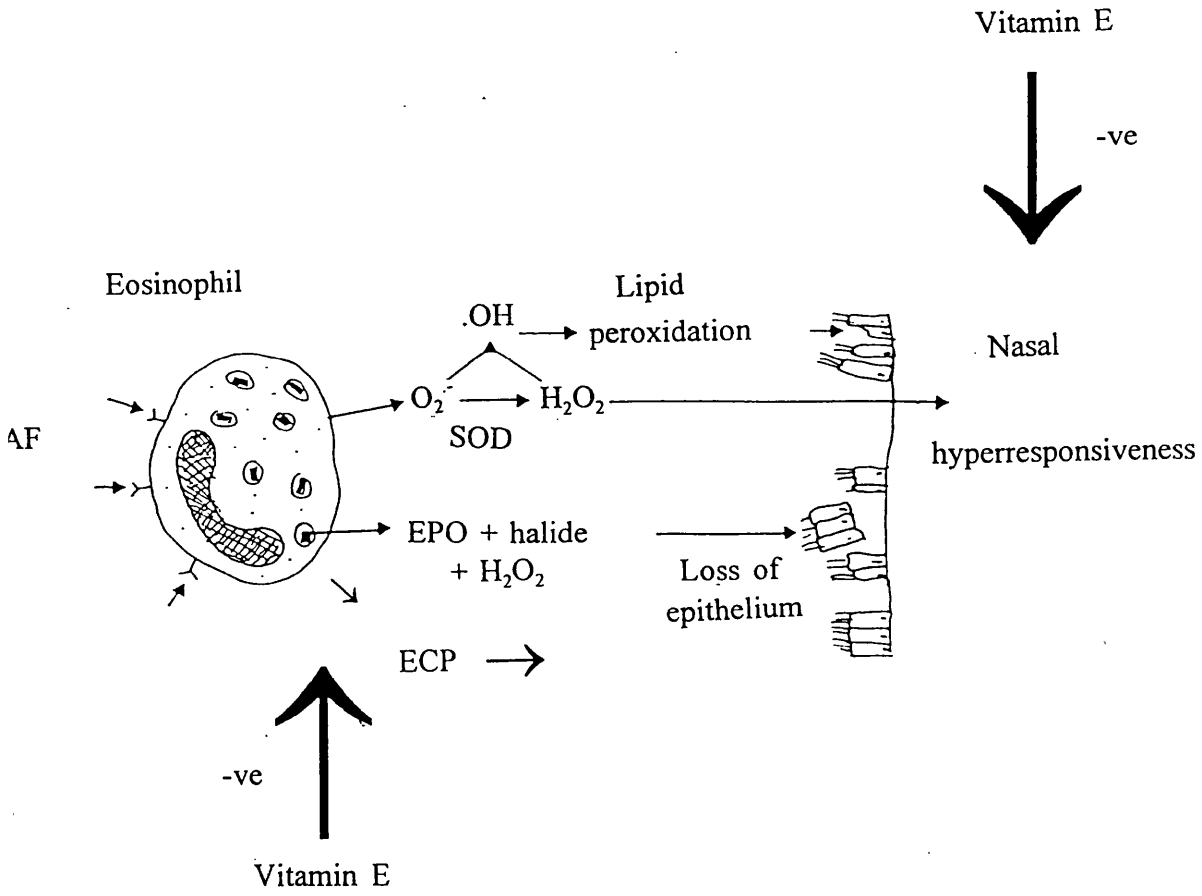


Diagram summarising the effects of PAF in the human nasal airway.

Additional references

Altman, L.C., Ayars, G.H., Baker, C. & Luchtel, D.L. (1993). Cytokines and eosinophil-derived cationic proteins upregulate intercellular adhesion molecule-1 on human nasal epithelial cells. J. Allergy Clin. Immunol. 92, 527-536.

Bajaj, P., Lawry, J., Shenton, G. & Rees, R.C. (1993). Interleukin-6 and tumour necrosis factor alpha synergistically block S-phase cell cycle and upregulate intercellular adhesion molecule-1 expression on MCF7 breast carcinoma cells. Cancer Lett. 71, 143-149.

Das, K.M., Squillante, L. & Robertson, F.M. (1993). Amplified expression of intercellular adhesion molecule-1 (ICAM-1) and M(r) 40K protein by DLD-1 colon tumor cells by interferon-gamma. Cell. Immunol. 147, 215-221.

Groves, R.W., Allen, M.H., Barker, J.N., Haskard, D.O. & MacDonald, D.M. (1991). Endothelial leucocyte adhesion molecule-1 (ELAM-1) expression in cutaneous inflammation. Br. J. Dermatol. 124, 117-123.

Gebremichael, I. & Leuenberger, P. (1992). Platelet-activating factor does not induce bronchial hyperreactivity in non asthmatic subjects. Respiration 59, 193-196.

Hizawa, N., Yamaguchi, E., Ohe, M., Itoh, A., Furuya, K., Ohnuma, N. & Kawakami, Y. (1992). Lack of linkage between atopy and locus 11q13. Clin. Exp. Allergy 22, 1065-1069.

Lai, C.K., Jenkins, J.R., Polosa, R. & Holgate, S.T. (1990). Inhaled PAF fails to induce airway hyperresponsiveness to methacholine in normal human subjects. J. Appl. Physiol. 68, 919-926.

Lympany, P., Welsh, K.I., Cochrane, G.M., Kemeny, D.M. & Lee, T.H. (1992a). Genetic analysis of the linkage between chromosome 11q and atopy. Clin. Exp. Allergy 22, 1085-1092.

Lympany, P., Welsh, K., MacCochrane, G., Kemeny, D.M. & Lee, T.H. (1992). Genetic analysis using DNA polymorphism of the linkage between chromosome 11q13 and atopy and bronchial hyperresponsiveness to methacholine. J. Allergy Clin. Immunol. 89, 619-628.

Rich, S.S., Roitman-Johnson, B., Greenberg, B., Roberts, S. & Blumenthal, M.N. (1992b). Genetic analysis of atopy in three large kindreds: no evidence of linkage to D11S97. Clin. Exp. Allergy 11, 1070-1076.

Spencer, D.A., Green, S.E., Evans, J.M., Piper, P.J. & Costello, J.F. (1990). Platelet activating factor does not cause a reproducible increase in bronchial responsiveness in normal man. Clin. Exp. Allergy 20, 525-532.