NASAL HYPERREACTIVITY

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PROEFSCHRIFT

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CONTENTS

LIST OF ABBREVIATIONS

BAL	bronchoalveolar lavage
CGRP	calcitonin gene-related peptide
ConA	concanaveline A
CTMC	connective tissue mast cells
D_2O	deuterium oxide
ECP	eosinophil cationic protein
EDN	eosinophil derived neutotoxin
EPO	eosinophil peroxidase
HDM	house dust mite
LTB_4,LTC_4,LTD_4,LTE_4	leucotriene B_4,C_4,D_4,E_4
MBP	major basic protein
MMC	mucosal mast cels
NANC	non-adrenergic non-cholinergic
NAR	nasal airway resistance
NEU	noon-equivalent units
NKA	neurokinin A
NPK	neuropeptide K
NPY	neuropeptide Y
PAF	platelet activating factor
PGD_2	prostaglandine D_2
PHI	peptide histidine-isoleucine
SP	substance P
TAME	tosyl-1-arginine methyl ester
T mast cells	tryptase mast cells
TC mast cells	tryptase/chymase mast cells
VIP	vasoactive intestinal polypeptide

PART I. INTRODUCTION AND LITERATURE

CHAPTER 1. INTRODUCTION

The subject of this thesis is nasal hyperreactivity or hyperresponsiveness in allergic and nonallergic rhinitis. Hyperreactivity represents an altered state of the upper or lower airways, resulting in an exaggerated response to non-specific stimuli encountered in the environment. Generally, hyperreactivity is a major feature of the disease leading to recurrent or chronic symptoms. In allergic rhinitis and asthma hyperreactivity will reinforce the response after allergen exposure.

Although it is known that patients with allergic and non-allergic rhinitis often react with symptoms to environmental non-specific stimuli, standardized and generally accepted tools for measurement of nasal hyperreactivity are not available.

Many investigators demonstrated a relation between hyperreactivity of the lower airways and IgE-mediated allergy, especially with respect to the link between hyperreactivity and late phase allergic reactions. However, in nasal allergy a relative backlog exists regarding insight into interactions between IgE-mediated reactions and nasal hyperreactivity.

This thesis focuses on answering two questions:

1. How to measure nasal hyperreactivity ?
2. What is the role and clinical significance of nasal hyperreactivity, especially in nasal allergy ?

After a review of the literature (part I, Chapter 2) the current methods of assessment of nasal hyperreactivity are analysed (part II). To this end, nasal challenges with various non-specific stimuli were performed in allergic and non-allergic rhinitis patients compared to healthy subjects (chapters 3,4 and 5).

Subsequently the clinical aspects of hyperreactivity in nasal allergy (part III) have been investigated. The relevance of hyperresponsiveness was analysed with respect to the daily symptoms of the patient and the activity of the nasal disease at the time of the test (Chapter 6). In addition the influence of natural allergen exposure on nasal challenge tests was studied (Chapter 7). Closely connected with this study is the investigation of late phase allergic reactions in relation to nasal hyperreactivity (Chapter 8). Finally a combined nasal hyperresponsiveness and allergy to avian antigens in a birdkeeper is reported in Chapter 9.

In Chapter 10 the experiments described in this thesis are summarised. In this chapter the methodology, pathophysiology and diagnostic value of measuring nasal hyperreactivity are discussed.

CHAPTER 2. LITERATURE

In this chapter the definition, underlying factors and measurement of nasal hyperreactivity will be reviewed. IgE-mediated allergy and the relation between allergy and hyperreactivity will be discussed. At the end the classification of rhinitis will be briefly discussed.

2.1 Nasal hyperreactivity

2.1.2 Definition

Hyperreactivity or hyperresponsiveness in the upper and lower airways refers to an increased sensitivity to non-specific stimuli or irritants. In case of hyperreactivity of the nasal mucosa the most prominent symptoms of rhinitis patients are sneezes, rhinorrhoea and nasal blockage on exposure to low doses of stimuli which do not induce symptoms in healthy subjects.

Hyperreactivity can be described as clinical feature characterised by occurrence of symptoms on exposure to daily-life stimuli such as dust particles, change of temperature, tobacco smoke, perfumes and paint smells. This clinical hyperreactivity has be to distinguished from hyperreactivity as determined by challenge with pharmacological or physical agents in the laboratory.

Thus, hyperreactivity can be regarded as a diagnostic feature entirely based upon an accurate history or as an abnormal dose-response relationship when provoking the nose or bronchi with pharmacological agents or physical stimuli. Dose-response relations in biology can be expressed in S-shaped curves (fig. 1). Hyperreactivity may be due to a shift of the dose-response curve to the left (fig. 1A), a steepened slope of the midportion (fig. 1B), a higher maximal plateau (fig. 1C) or an increased baseline level (fig. 1D). A combination of these changes is also possible.

The concept of nasal hyperreactivity as measured by nasal challenge tests is derived from earlier studies in bronchial asthma, which described the phenomenon of bronchial hyperreactivity. In general the term bronchial hyperreactivity indicates an increased responsiveness of the lower airways to challenges with non-specific stimuli, as measured

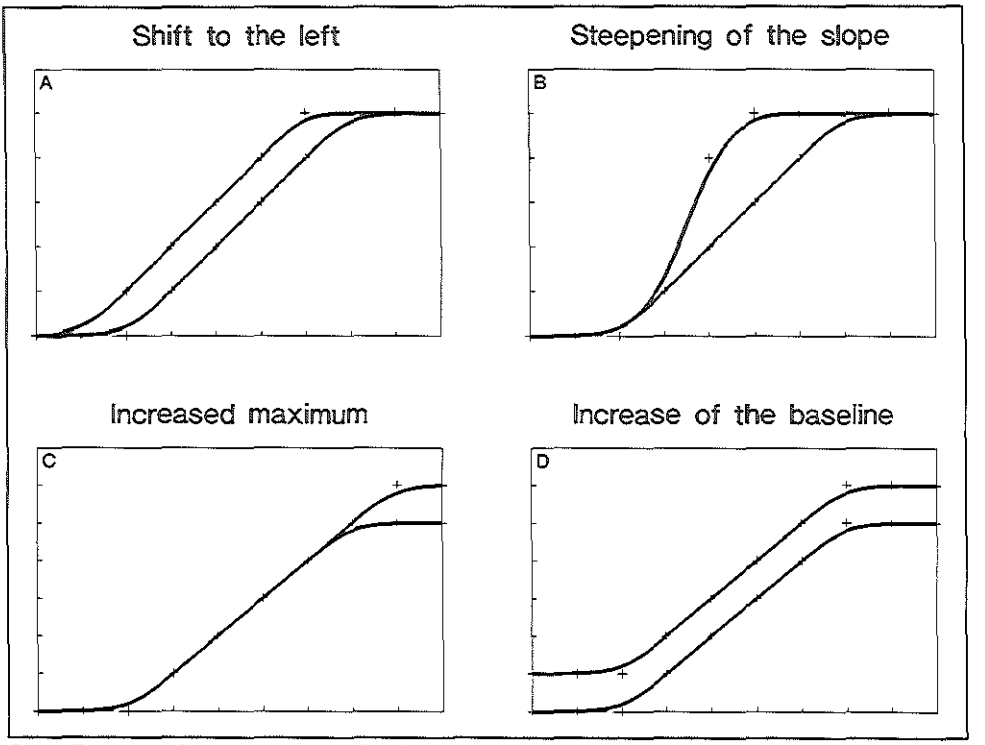

fig. 1. Examples of dose-response relations in nasal and bronchial hyperreactivity.

by alterations in lung function. Commonly used stimuli are histamine and acetylcholine or methacholine. While investigating the systemic effects of histamine (Weiss et al. 1932) and acetylcholine (Dale 1914) in man, it was noted that these agents precipitated asthmatic attacks in asthma patients. By comparison with healthy subjects and by determination of the dose required to induce a bronchial reaction, a dose-dependent hyperresponsiveness to histamine and acetylcholine could be demonstrated in asthmatic patients (Curry 1946, Curry 1947, Tiffeneau 1958). Since then research into bronchial hyperreactivity has been extended. In our country also de Vries and coworkers (de Vries 1987) have increased the understanding of bronchial hyperresponsiveness.

In analogy with these experiments rhinitis patients and healthy subjects underwent nasal challenge tests with veratrine (van Lier 1960), histamine (Grobler 1966) and methacholine (McLean 1977, Borum 1979).

However, it will be clear that nasal and bronchial hyperreactivity cannot be compared easily when taking the differences in the target organs into account. The bronchial

response is dominated by constriction of smooth muscle whereas the nasal response is characterised by induction of the sneeze reflex, stimulation of glands and dilatation of vessels.

Determination of hyperreactivity by nasal provocation tests assumes a relation between this form of hyperreactivity and clinical hyperreactivity. A few studies have demonstrated that nasal reactivity to histamine or methacholine correlates with the severity of rhinitis (Grobler 1966, Asakura et al. 1984). However, the classification of the degree of rhinitis was rather rough. Grobler used 3 classes (giving nasal blockage, sneezes and secretion one point each). Asakura divided the severity of symptoms into three catagories mild, moderate and severe. A definition of these three catagories was not given. Nasal sensitivity to non-specific stimuli has not been studied in relation to more quantitatively defined symptom scores. Moreover the above mentioned association between clinical hyperreactivity and hyperreactivity determined by nasal challenge tests has never been investigated.

2.1.3 Mechanisms of nasal hyperreactivity

2.1.3.1 Reactions of the nasal mucosa to stimulation

In order to understand the reaction of the nose to stimulation of the mucosa a few aspects of nasal anatomy and physiology will be discussed (Mygind & Anggard 1984, Eccles 1982, Eccles 1987a, Eccles 1987b). Anatomically the external nose is composed of the bony pyramid, the upper lateral cartilages, the lower lateral cartilages and the septum which divides the nose into two compartments. The internal nasal cavity is much larger and extends from the external nares anteriorly to the posterior choanae.
The lateral wall of the nasal cavity contains the three turbinates. The bones and cartilage that form the framework of the nose are covered by periosteum, perichondrium and a mucous membrane.
The mucous membrane shows regional differences: the mucosa is thin on the nasal septum whereas the inferior turbinate is covered by a thick, densely vascularised mucous membrane. The epithelial lining is composed mostly of pseudostratified columnar ciliated cells. The vasculature of the nose consists of capillaries just beneath the epithelium and

around glands, and deeper in the lamina propria: precapillary resistance vessels, venules, venous erectile tissue (venous sinusoids with smooth muscle), and arteriovenous anastomoses. The filling of venous erectile tissue determines the state of nasal congestion and the resistance to nasal airflow. Subepithelial glands containing both serous and mucous cells are responsible for nasal secretion. In addition goblet cells lying between surface columnar cells contribute to the secretion.

Mast cells are found in the lamina propria but rarely in the epithelium. Lymphocytes (predominantly T-lymphocytes) are found both in the epithelium and lamina propria. CD1+ cells, probably Langerhans cells have recently been demonstrated in the nasal epithelium (Fokkens et al. 1990).

The nerve supply of the nose consists of sensory nerves derived from the ophthalmic and maxillary divisions of the trigeminal nerve, postganglionic parasympathetic fibres for the nasal blood vessels and glands derived from the sphenopalatine ganglion and sympathetic nerve fibres which follow the carotid artery after leaving the stellate ganglion in the lower neck.

Stimulation of sympathetic nerve fibers will lead to release of neurotransmitters which act on α- and β- adrenergic receptors. These receptors have been found in homogenates of the nasal mucosa (Ishibe et al. 1983, Konno et al. 1987a, van Megen 1989). They are responsible for the regulation of nasal blood flow (Andersson & Bende 1984). Stimulation of parasympathetic nerves will act on muscarinic receptors located on nasal glands (van Megen 1989).

The nature of the stimulus determines the character of the nasal response after stimulation of the mucosa. Challenge experiments in the laboratory have shown that glands are stimulated by acetylcholine (Grobler 1966) or methacholine (Borum 1979). This effect is mainly a consequence of direct stimulation of nasal glands as methacholine induces nasal secretion even after vidian neurectomy (Konno et al. 1983, 1987b). Analysis of methacholine-induced nasal discharge demonstrated that transudation was not the source of the nasal secretion (Konno et al. 1983, 1987b, Raphael et al. 1988). Histamine leads to increase of nasal airway resistance (NAR), secretion and sneezes (Mygind 1982). The increase of NAR is considered to be due to a direct effect on nasal vasculature whereas secretion is mediated by central reflexes. Support for these

assumptions was delivered by Mygind et al. (1983a) and Konno et al.(1983,1987b). One-sided nasal histamine challenge induced only ipsilateral increase in NAR without effect on the contralateral side (Mygind et al. 1983a). Moreover this effect was not blocked by vidian neurectomy (Konno et al. 1983, 1987b). One-sided nasal challenge with histamine induced secretion, which could be abolished almost completely by vidian neurectomy implicating the involvement of central reflexes (Konno et al. 1983, 1987b). In contrast to these studies, biochemical analysis of histamine-induced rhinorrhoea indicated that both reflex-mediated glandular secretion and plasma leakage may contribute to the nasal discharge (Raphael et al. 1989).

Most research into nasal reflexes has been performed in animal studies. Stimulation of sensory nerve endings will lead to a local axon reflex and to the release of the neurotransmitter substance P (SP) which induces vasodilatation and increased vascular permeability with subsequent oedema (Lundblad et al. 1983a, 1983b; Uddman & Anggard 1987). Apart from SP structurally related tachykinins such as neurokinin A (NKA) and neuropeptide K (NPK) have been found in these nerves (Stjärne et al. 1989a). Additionally, calcitonin gene-related peptide (CGRP) responsible for relaxation of vascular smooth muscle, has been demonstrated (Stjärne et al. 1989a). Stimulation of sensory nerve endings will also lead to a central reflex through afferent fibres, the central nerve system and efferent parasympathetic and sympathetic nerves (Malm et al. 1987). Parasympathetic activation will result in nasal secretion by means of the neurotransmitter acetylcholine. In the nose the parasympathetic pathways are closely related to the non-adrenergic non-cholinergic (NANC) system. Consequently, activation of the parasympathetic system will also result in release of the neurotransmitter vasoactive intestinal polypeptide (VIP) and the structurally related peptide histidine-isoleucine (PHI) inducing vasodilatation and decrease of nasal patency (Anggard et al. 1983, Lundberg et al. 1984, Uddman & Anggard 1987). Sympathetic fibres regulate the vascular tone in the nose. Stimulation leads to release of noradrenalin and neuropeptide Y (NPY) with subsequent vasoconstriction and widening of the nose (Uddman & Anggard 1987). Blockage of these pathways will result in a decrease of nasal patency (Richardson & Seebohm 1968). Apart from the previously mentioned reflexes a sneeze reflex through somatomotor nerves can be induced. Sneezing is a respiratory reflex (an expiratory phase preceded by an inspiratory phase and characterized by an explosive blast of air

escaping through the nasal and oral cavities). Actually, sneezing is a part of a generalised reflex response including twitching of the nose and face, blinking and tears (Cole 1982, Eccles 1982).

2.1.3.2 Factors underlying nasal hyperreactivity

Several hypotheses have been advanced with respect to the mechanisms underlying hyperreactivity in allergic and nonallergic rhinitis (Mygind & Anggard 1984).

1. *Increased epithelial permeability* would ease the accessibility for stimuli to sensory nerve endings, vessels and nasal glands. Evidence for this hypothesis is circumstantial as data on this subject are derived from studies in allergic and non-allergic rhinitis - diseases which can be dominated by nasal hyperreactivity. However, investigations of a direct relationship between epithelial permeability and nasal hyperreactivity have never been performed.

A better penetration of the mucosa was demonstrated in allergic rhinitis patients compared with healthy subjects, measured by application of topical albumin-^{125}I to the nasal mucosa (Buckle et al. 1975). In allergic rhinitis alterations in the nasal mucosa (destruction of the basal membrane, decrease in collagen, infiltration of eosinophils) were observed after repeated allergen challenges (Connell 1968b). Jahnke (1981) showed distended endothelial cells after the first few minutes of the allergic reaction and pronounced interstitial oedema within ten minutes. In allergic patients with perennial symptoms the epithelial layer shows loosening of the cells, deformity of the cell contour and transudation. Intercellular spaces are dilated (Jahnke 1978). However, in recent studies destruction of the nasal mucosa in allergic patients could not be demonstrated (Fokkens et al. 1990, Lozewick et al. 1990). The discrepancies between these studies could be due to patient selection. Two studies concern alterations after allergen challenges (Connell 1968b, Jahnke 1981), one study has perennial allergic rhinitis as its subject (Jahnke 1978) and two studies focuses on seasonal allergic rhinitis patients (Fokkens et al. 1990, Lozewick et al. 1990). Spector et al. (1980) demonstrated a general loss of surface epithelium and columnar cells in the nasal mucosa of non-allergic rhinitis subjects compared with subjects without rhinitis.

In conclusion, some of the studies mentioned are suggestive of increased epithelial

permeability. However, to investigate the direct relationship between nasal hyperreactivity and epithelial permeability other study designs are required. Nasal hyperreactivity has to be quantified and the outcome to be correlated with epithelial destruction and increased permeability.

2. *An increased sensitivity of sensory nerve endings (irritant receptors)* would induce an exaggerated response to normal stimuli. No firm data are available yet to confirm this theory.

3. *Changed modulation of afferent impulses in the central nervous system* has been proposed as a cause of hyperreactivity. The only data to support this hypothesis have been put forward by Eccles and Lee (1981). They demonstrated in cats that stimulation of the hypothalamus leads to vasoconstriction in the nose and they proposed that prolonged exposure to stress could result in failure of the hypothalamic control over sympathetic innervation. This condition would lead to a relative dominance of the parasympathetic system over the sympathetic pathways. Again no firm data are available on this subject.

4. *Changed number or sensitivity of cell receptors* could underlie nasal hyperreactivity. In bronchial asthma much research has been performed into cell receptors. The theory that a β- adrenergic blockade is responsible for bronchial hyperreactivity and asthma (Szentivanyi 1968), has attracted much attention. The hypothesis that β-adrenergic blockade is a cause and not a consequence of bronchial hyperresponsiveness and asthma has been questioned by Koëter and Meurs (1984). They showed that in allergic asthma lymphocyte β-adrenergic receptors decrease after an asthmatic attack, and that this decrease is accompanied by an increase in bronchial hyperresponsiveness to the β-adrenergic antagonist propranolol (Koëter & Meurs 1984).
With respect to nasal hyperreactivity little is known of cell receptors. An increased number of muscarinic receptors was found in patients with nasal allergy (Konno et al. 1987a). As allergic patients are hyperresponsive to methacholine (Druce et al. 1985, Konno et al. 1987b), it is possible that the increased number of muscarinic receptors plays a role in the exaggerated nasal discharge seen in allergic rhinitis patients. Also

increase of muscarinic receptors in non-allergic vasomotor rhinitis patients might correspond with observations from challenge studies. Stjärne et al. (1989b) exposed normal subjects and vasomotor rhinitis patients with symptoms of rhinorrhoea and sneezing to capsaicin - an agent able to induce severe irritation, sneezing and hypersecretion by release of substance P from sensory nerves. The secretory response which could be blocked by pretreatment of anticholinergic drugs, was found to be greater in the patients, whereas the irritation of the nose was the same both in the healthy persons and the patients with vasomotor rhinitis. These differences in effect were attributed to a change at the cholinoceptor site on the glandular cell and/or glandular hypertrophy, with an equal absorption of the test agents in healthy subjects and patients. Konno et al. (1987b) revealed a decrease of α1- and β-receptors in the nasal mucosa of allergic rhinitis patients compared with non-allergic patients with sinusitis, an observation previously demonstrated by Ishibe et al. (1983). Although the clinical significance of these findings is not known, it is possible that a decreased density of α1-adrenergic receptors in the nasal mucosa will facilitate vasodilatation and swelling of the nasal mucosa in nasal allergy. However, in contrast to the studies of Konno and Ishibe, van Megen (1989) was unable to detect a decrease in α-adrenergic receptors in the nasal mucosa of allergic patients. She demonstrated an increased sensitivity of muscarinic receptors and a decrease in number of β-adrenergic receptors in the nasal mucosa of allergic patients, but these findings were considered to be of minor importance in the pathogenesis of allergic rhinitis, as these shifts in sensitivity and number of cell receptors were too small to explain the complex allergic reaction. The discrepancy between the findings of the Japanese studies and the experiments of van Megen could be due to differences in study design. Van Megen investigated allergic rhinitis patients without clinical manifestations at the time of the experiments. The Japanese studies were performed using patients with allergic perennial rhinitis. No information was provided on the severity of symptoms during the investigations. In addition it is possible that the difference in control subjects (non-allergic patients with sinusitis in the Japanese studies and predominantly healthy subjects in the study of van Megen) is responsible for the contradictory results.

All these studies focuses on patients with allergic rhinitis. The degree of non-specific nasal hyperreactivity was not determined in the investigations. So, the above mentioned

studies do not solve the question whether nasal hyperreactivity itself is associated with alterations in muscarinic or adrenergic receptors. For instance, it is possible that an association exists between the methacholine-induced nasal response and an increase in number or sensitivity of muscarinic receptors of the nasal mucosa.

5. *Mediator release after non-specific stimulation* of the nose has been demonstrated in patients who experienced nasal symptoms after exposure to cold air. It was shown that in this subgroup of patients nasal provocation with cold, dry air (CDA) will lead to mast cell activation and consequent mediator release (Togias et al. 1985, 1986, 1987, Iliopoulos et al. 1988). This mediator release appears to differ from IgE dependent mediator release as azatadine, a tricyclic antihistamine capable of inhibiting the nasal reaction to allergen, does not affect the CDA-induced mediator release in contrast to the allergen-induced mediator release (Togias et al. 1987). Detailed information on mast cells and mediator release is given in chapter 2.2.2 .
The assumption has been made that mast cells degranulate by an increased osmolarity of the tissue due to evaporation. This is in agreement with the observation that CDA-induced mediator release is associated with an elevated osmolality in situ (Togias et al. 1988).
In the above mentioned studies an unphysiological method was used to stimulate the nasal mucosa: 187.5 litres of cold dry air was blown into the nose within fifteen minutes. It is therefore questionable whether these experiments reflect daily-life situations.

In summary, several mechanisms have been put forward as an explanation for nasal hyperreactivity. The major drawback of human studies is that these investigations are focused on diseases such as allergic rhinitis but not on nasal hyperreactivity itself. So far it is not known whether one or more of these mechanisms play a role in the pathogenesis of nasal hyperreactivity. As allergen exposure leads to an increase of nasal hyperreactivity within 24 hours (Linder et al. 1987, Andersson et al. 1989), it is unlikely that alterations in the central nervous system are responsible for such rapid changes. Changes in nasal permeability or receptor function of the nasal mucosa after allergen exposure are more likely to explain this hyperresponsiveness. However, to

answer these questions research has to be performed into alterations in nasal permeability or changes in receptor function in connection with nasal hyperresponsiveness. It is conceivable that no single abnormality can fully explain nasal hyperresponsiveness. Moreover it is possible that distinct mechanisms are important in different patient populations depending on the nature of the nasal disease.

2.1.4 Measurement of nasal hyperreactivity

2.1.4.1 Introduction

Attempts to discriminate between patients and healthy subjects by means of challenge with non-specific stimuli have led to conflicting results (Asakura et al. 1984, Borum 1979, Clement et al. 1985, Corrado et al. 1986, Filiaci & Zambetti 1983, Guercio et al. 1979, McLean et al. 1977, Mullens et al. 1989, Okuda et al. 1983a). These studies concerning nasal hyperreactivity differ from each other in the provocation technique, in the way of assessing the symptoms and in selection of the patient population. When discriminating rhinitis patients from healthy subjects, a considerable overlap is often encountered.

2.1.4.2. Nasal provocation

Application methods

Several methods have been used to apply provocation agents. Dry systems, challenge solutions and gases have been applied in nasal challenge (see table I). It is not possible to pronounce upon the best method of application as systematic comparisons of delivery systems have not been made. Theoretically a delivery system without non-specific effects would be preferable. Protocols which may irritate the nose such as paper disks, cotton wool or the delivery by pipettes require challenges with control solutions.

Assessment of the nasal response

No standardised methods are available for assesment of the nasal response. Depending on the chemical or physical nature of the challenge, the nose may react with nasal blockage, sneezing or secretion.

Methods of assesment include use of visual analog scales (Linder 1988) or combined

Table I. Application methods

Dry systems:		
Paper disks impregnated with the challenge substance		Okuda et al. 1983a
Cotton wool impregnated with challenge substance		Pelikan 1977
Whole pollen grains delivered in the form of dry powder		Naclerio et al. 1983
Challenge solutions:		
Solutions delivered by	nebulizer	Clement et al. 1985
	mechanical pump spray	Pipkorn 1982
	pipettes	Mygind et al. 1986
Vapourised systems delivering:		
gases such as NH_3		Mathews et al. 1979
cold air		Togias et al. 1985

symptom scores taking nasal blockage, secretion and sneezes (Borum et al. 1980) or a combination of all signs and symptoms (Lebel et al. 1988) into account. A simple method used in clinical practice is the judgement by rhinoscopy, which can be as informative as more sophisticated methods such as measurement of NAR (Wihl & Malm 1985a). Fiberoptic rhinoscopy might also estimate nasal patency accurately (Zedalis et al. 1989).

It is possible to quantify nasal blockage by monitoring the pressure changes in the nasopharynx (posterior rhinomanometry) or on one side of the nasal cavity (anterior rhinomanometry) (Clement 1984a). Anterior rhinomanometry takes place during normal breathing (active rhinomanometry) or during delivery of a constant airflow through a nozzle (passive rhinomanometry) (Clement et al. 1978). Posterior rhinomanometry has the advantages of measurement of total nasal resistance, without the nasal deformation seen in anterior rhinomanometry. However, at present the technique of the active anterior rhinomanometry is advised for measurement of NAR (Clement 1984b), since with the former technique 30-50% of the patients cannot be measured. Although not the most sensitive technique, passive anterior rhinomanometry (PAR) has been recommended by Clement & Kaufman (1984c) as the easiest and most suitable technique for measuring nasal resistance during nasal provocation tests.

Other ways of determination of nasal patency include nasal expiratory or inspiratory peak flow measurement (Gleeson et al. 1986, Wihl & Malm 1988). Recently an acoustic method has been put forward for measuring the geometry of the nasal cavity (Hilberg et al. 1989). Compared with anterior rhinomanometry low coefficients of variation were obtained (2% versus 15%).

Methods available to measure secretion include collection of drips from the patient's nose in a tube or syringe (Borum 1979), suction of secretion (Druce et al. 1985) and the use of filter paper to absorb secretion (Mygind 1978).
Sneezes after challenge are simply counted (Okuda et al. 1983a).

The choice of methods to determine nasal patency will depend on the purpose of the test. When accurate information on nasal patency is required, methods such as active anterior and posterior rhinomanometry are appropriate. The use of active anterior rhinomanometry has the advantage that this method is recommended by the European Standardisation Committee (Clement 1984b). The use of standardised methods permits a better comparison between studies performed in different centres. Nasal challenge tests require less sensitive methods such as PAR or nasal peak flow as in these situations large increases in NAR or decreases in flow have to be achieved to determine a nasal threshold value: for instance the concentration of a test agent required to induce a doubling of NAR can be considered as the threshold or endpoint concentration. In addition both PAR and peak flow are easily applied in clinical practice, and they are useful in testing children. When only qualitative information is required (i.e positive or negative reactions) clinical judgement of the nasal response by rhinoscopy would be informative.
New methods such as fiberoptic rhinoscopy or acoustic rhinometry are of interest. In particular acoustic rhinometry provides information on circumscribed reactions of the nasal mucosa, which do not affect nasal airway resistance. Thus, this method seems to be more sensitive than rhinomanometry (Lenders & Pirsig 1990).
To sum up, a wide variety of methods to assess the nasal response is available to the investigator. The reproducibility of these methods has rarely been the subject of investigation. Table II gives an overview of this topic. The reproducibility of nasal challenge tests has only been reported in a few studies (Borum 1979, Borum et al. 1980, Clement et al. 1985, Corrado et al. 1987). In allergen challenge the reproducibility increases when attention is paid to a combination of symptoms (Borum 1980 et al.). The use of combined symptoms appeared to be more suitable in monitoring immunotherapy than isolated symptoms did (Wihl 1986).

Table II. Methods of assesment of the nasal response

	Reproducibility
a. Subjective measurement	
Clinical judgement	not known
Visual analog scale	not known
Combined scores	not known
b. Nasal patency	
Rhinomanometry	
PAR	c.v.= 38% in repeated measurements of the resting NAR; individual fluctuation in repeated histamine challenges (Corrado et al. 1987), without fluctuation in group responses to histamine (Clement et al. 1985, Corrado et al. 1987).
AAR	c.v.= 65% in allergen challenge (Borum et al. 1980)
Peak flow	c.v.= 38% in repeated measurements of the resting NAR (Taylor et al. 1973); not known in challenge protocols.
Acoustic rhinometry	c.v.= 2% in repeated measurements (Hilberg et al. 1989); not known in nasal challenges
Fiberoptic rhinoscopy	c.v.= 7.93-21.7% in repeated measurements (Zedalis et al. 1989); not known in nasal challenge.
c. Secretion	c.v.= 41% in allergen challenge (Borum & Mygind 1980) c.v.= 25% in methacholine challenge (Borum 1979)
d. Number of sneezes	c.v.= 34% in allergen challenge (Borum & Mygind 1980)
e. Combination of NAR, sneezes and secretion	c.v.= 23% in allergen challenge (Borum & Mygind 1980)

c.v. = coefficient of variation

Several methods employed to measure nasal patency have a high coefficient of variation (table II). No significant fluctuations are demonstrated in NAR measurements after histamine challenge, however this observations holds true of groups only (Clement et al.1985, Corrado et al. 1987). In contrast, a marked variability is seen in the nasal response of the individual (Corrado et al. 1987). The reproducibility of nasal provocation may be affected by the considerable spontaneous change of NAR (Kumlien & Schiratzki 1977). Differences in baseline values will also increase the variability of provocation-response relationships (Mygind & Borum 1983b). Moreover, other effects of the test agent, such as sneezing and rhinorrhoea might influence nasal patency and its measurement.

Surprisingly, a study of the variability of the different actions of histamine has never been performed, although histamine also induces an increase in NAR, rhinorrhoea and sneezing.

Provocation with histamine

Histamine application in the nose will induce an increase of nasal airways resistance and a secretory response and will elicit the sneeze reflex (Mygind 1982). In 1966 Grobler (Grobler 1966) could distinguish patients from healthy subjects by topical application of histamine in the nose. The former group showed increased responsiveness to histamine. Since then several attempts have been made to discriminate between healthy subjects and both allergic (McLean et al. 1977, Guercio et al. 1979, Corrado et al. 1986) and non-allergic (Clement et al. 1985) rhinitis patients by use of nasal provocation with histamine. Unfortunately most investigators encounter a considerable overlap between the healthy subjects and patients (Mygind & Borum 1983b, Pipkorn 1989a). McLean et al. (1977) and Guercio et al. (1979) found no difference between controls and allergic rhinitis patients without symptoms at the time of the challenges. Other investigators (Corrado et al. 1986, Mullens et al. 1989, Clement et al. 1985) challenged rhinitis patients with symptoms and demonstrated hyperresponsiveness to histamine in this group: Corrado et al. (1986) could distinguish between rhinitis patients allergic to house dust mite with severe symptoms and healthy subjects. In one study a clear-cut difference between healthy subjects and rhinitis patients allergic to ragweed pollen during the season could be seen when tested during the season (Mullens et al. 1989). Clement et al. (1985) found a statistically significant but small difference between non-allergic rhinitis patients and healthy subjects, using active and passive anterior rhinomanometry. One-sided measurements of the NAR and determination of a PD_{25} (the dose required to induce 25% increase in NAR) appeared to be superior in separating non-allergic rhinitis patients to measurement of the total NAR and determination of a PD_{100} (Heyning et al. 1989). All studies above were performed with the increase of airway resistance as a parameter for the nasal response.

In contrast Okuda et al. (1983a) demonstrated hyperresponsiveness to histamine in allergic rhinitis patients with symptoms at the time of the challenges by counting the number of sneezes after application of a paper disk soaked in histamine, a technique not used in Europe or the USA.

At present nasal challenges with histamine are not recommended for clinical practice because of the large overlap between patients and healthy subjects. However, they are useful research tools in the evaluation of drugs such as nasal decongestants (Britton et

al. 1978, Corrado et al. 1987) and corticosteroids (Pipkorn 1982), as in these studies patients are used as their own controls.

Provocation with methacholine

The first experiments with acetylcholine applied intranasally were performed by Grobler (1966) who tested healthy subjects only. McLean et al. (1977) and Borum (1979) tested both healthy subjects and rhinitis patients with the stabler methacholine. In nasal allergy hyperresponsiveness to methacholine was shown by Druce et al. (1985) and Konno et al. (1987a), but not by McLean et al. (1977). It was also demonstrated by Borum (1979) that methacholine can induce an increased secretory response in perennial non-allergic rhinitis. A second study confirmed the existence of hyperreactivity to methacholine in perennial rhinitis of non-allergic origin (Filiaci et al. 1983). In another study nasal hyperresponsiveness to methacholine could only be demonstrated in allergic subjects, but not in non-allergic rhinitis patients (Asakura et al. 1984).

All these studies differ in their design. McLean et al. (1977) and Druce et al. (1985) challenged rhinitis patients allergic to ragweed pollen outside the season. Konno et al. (1987) studied patients with perennial allergic rhinitis. Borum (1979) challenged highly selected non-allergic patients, as the patients had symptoms for 6 hr a day and used, on average, 27 paper handkerchiefs a day. The severity of symptoms in the study of Filiaci et al. (1983) and Asakura et al. (1984) is not known.

The way of assessment of the nasal response also varies in these studies. McLean et al. (1977) and Filiaci et al. (1983) measured NAR, Druce et al. (1985) determined the amount of albumin in secretion, Borum (1979), Asakura et al. (1984) and Konno et al. (1987) measured the amount of secretion.

Again, none of these methods is recommended for daily clinical practice.

Provocation with other agents

Nasal challenges have been performed with substances such as polymyxine B which increases NAR (McLean et al. 1978), platelet activating factor (PAF) which induces vasoconstriction (Pipkorn et al. 1984), substance P which causes both nasal (NAR increase) and systemic symptoms such as cutaneous flushing (Wolf et al. 1987, Devillie et al. 1988) and NH_3 which induces nasal blockage (McLean et al. 1979).

Physical stimuli have been used. Cold dry air causes nasal symptoms and can lead to mast cell degranulation (Togias et al. 1985, 1986). Challenge with hyperosmolar fluids will induce a higher increase in the NAR of patients compared with controls (Krayenbuhl et al. 1988) and mediator release in healthy subjects (Silber et al. 1988). Except for challenges with hyperosmolar stimuli (Krayenbuhl et al. 1988) and SP (Devillie et al. 1988), none of the studies provides a method to distinguish patients from controls and therefore suitable to measure nasal hyperresponsiveness.

Methodological problems in nasal challenge

Several methodological problems are encountered in challenging the nose (Mygind & Borum 1983b).

Firstly, problems concern on the delivery of substances. The challenge technique by itself can lead to a reaction of the nasal mucosa. McLean et al. (1976) showed that provocation with phosphate buffered saline (PBS) will lead to increase in NAR. Preservatives in nose sprays such as benzalkonium chloride can also act as irritants (Mygind & Borum 1983b). Application of cotton wool (Pelikan et al. 1977, Pelikan 1978) and paper disks (Okuda et al. 1983a) can irritate the nose by themselves. The application by a Carlsberg pipette (Mygind 1986) requires rhinoscopy and is not possible without touching the mucous membrane. In all these cases control challenges have been performed in order to distinguish the specific action of the test agent from the non-specific effects of delivery.

Secondly, problems concern the measurement of the response. When measuring NAR the initial baseline will influence the level of increase after provocation as the system has a ceiling in the form of total blockage. Septal deviation will influence one-sided resistance measurements. One-sided resistance measurements will also be influenced by the existence of the nasal cycle, the phenomenon of cyclic one-sided increase and contralateral decrease in nasal patency (Eccles 1987a). The latter influences can be diminished by measurement of the total nasal resistance which is not susceptible to these cyclic changes (Eccles 1987a). Measurement of the NAR by passive anterior rhinomanometry has the disadvantage that the airstream required to produce a pressure gradient could theoretically on the one hand irritate the nose and on the other clear the nose from secretion. In addition the concentration of test substance can have an effect on

the character of the nasal response. The deposition of a small amount of a concentrated solution on a small area of the nasal mucosa will favour reflex-induced symptoms (sneezing, hypersecretion) while a diluted solution distributed over a large area will favour an increase in NAR (Mygind & Borum 1983b).

2.1.4.3 Bronchial hyperresponsiveness. Similarities and differences with nasal hyperreactivity

Bronchial provocation tests with non-specific stimuli to determine bronchial hyperresponsiveness have been used long before the first nasal challenge tests. Bronchial challenge tests with histamine or methacholine have been advocated as useful tools in clinical practice as they can discriminate between asthmatic patients and healthy subjects (Cockroft et al. 1977). However, in other studies a considerable overlap in hyperresponsiveness between asthmatic patients and healthy subjects has been observed (Popa & Singleton 1988, Casale et al. 1988). Moreover, in epidemiological studies it has been recognized that the prevalence of bronchial asthma is much lower than that of bronchial hyperresponsiveness (Woolcock & Peat 1987).

The main difference between nasal and bronchial challenge lies in the degree of overlap between patients and healthy subjects. The mean PC_{20} for histamine is 30-40 times lower in asthmatic patients who are using regular medication, compared to healthy subjects (Cockroft et al. 1977), whereas a two- to fourfold difference in nasal endpoint concentrations for histamine has been observed in rhinitis patients compared with healthy subjects (Clemens et al. 1985, Heynig et al. 1990).

Perhaps the most important difference between nose and bronchi is the absence of smooth muscle in the nose. The presence of bronchial smooth muscle might magnify the differences in hyperresponsiveness between patients and healthy subjects.

2.1.4.4 Conclusion

Nasal hyperreactivity as a clinical feature is characterised by a history of complaints under certain conditions such as exposure to tobacco smoke, paints smell, change of temperature etc. Its pathophysiology and that of its counterpart measured in the laboratory is poorly understood. In contrast, the knowledge of bronchial hyperresponsiveness has grown in the past few decades, although still various

pathofysiological explanations are being put forward as mechanisms responsible for this hyperresponsiveness (Sterk & Bel 1989). The methods used to measure bronchial hyperresponsiveness are defined better than the tests used to determine nasal hyperreactivity.

Despite the differences between bronchial and nasal tissue with their distinct response patterns to stimuli, nasal provocation tests with histamine and methacholine - agents commonly used in bronchial challenge tests - are often performed. Amazingly, a systematic approach to the methodology of measurement of nasal hyperreactivity does not underlie this transfer of pulmonological methods to the nasal situation.

Several other reasons to explore and evaluate the applicability of nasal provocation tests with histamine and methacholine can be advanced. The choice of histamine can be justified by its capacity to induce the nasal symptoms of sneezing, rhinorrhoea and obstruction which normally trouble rhinitis patients. From this point of view most studies on nasal challenge tests with histamine are inadequate as they only focus on the increase in NAR induced by this agent. A comparison of the different symptoms after challenge to determine the best way of assesment has never been made.

In addition, the reproducibility of these symptoms has to be evaluated.

Challenge with methacholine may provide additional information as this agent stimulates the nasal mucosa differently (by stimulation of glands) from histamine (by stimulation of sensory nerve endings and blood vessels). By comparison of both methods the preferable technique can be selected.

The concept of nasal hyperreactivity does not necessarily imply that a single method is applicable in all types of rhinitis. Gökemeyer and de Vries (Gökemeyer 1976) demonstrated that patients with asthma differ from patients with chronic bronchitis in their response pattern to various stimuli (histamine, methacholine, propranolol and fog). In this study a blockade of the β-adrenergic tone in the asthma group and an increased cholinergic activity in the bronchitis group were demonstrated. Although these data cannot be transferred to nasal disease, the underlying idea of a different pathophysiology in various patient groups reflected in different response patterns to exogenous stimuli may be important when studying nasal hyperreactivity in different patient groups.

Another question remains unanswered. Despite the various studies on nasal hyperreactivity, it is not known whether the nasal response after non-specific stimulation

really reflects the 'clinical hyperresponsiveness' assessed by an acurate history. Because of these white spots in the area of nasal hyperreactivity, in this thesis the methods to determine nasal hyperresponsiveness are not only evaluated with respect to their capability to distinguish patients from healthy subjects.

By use of several stimuli (histamine, methacholine and phentolamine) in various patient groups the occurrence of response patterns has been studied. In addition histamine challenge tests have been evaluated with respect to the association with 'clinical hyperreactivity' and severity of symptoms.

2.2. Allergy

2.2.1. Introduction

Hyperreactivity of the lower airways has extensively been studied within the context of allergic disease. Also, in rhinitis most studies have been focuses on patients with an allergy to inhalant allergens. Inflammation is a characteristic of allergic reactions. It has been assumed that this inflammation plays a part in the development of hyperreactivity. In order to understand the interaction between allergy and hyperreactivity the allergic reaction with its consequences will be discussed in more detail.

2.2.2 Definition

Allergy has been defined as 'untoward physiologic events mediated by a variety of different immunologic reactions' (Middleton et al. 1978). This definition implies the acceptance of three criteria necessary for the definite diagnosis of an allergic state: (1) identification of the exogenous antigen, (2) establishment of a causal relationship between exposure to the antigen and occurrence of the disease, and (3) demonstration of an immunologic mechanism involved in the illness.

For clinical purposes Voorhorst (1962) has defined allergy as an altered sensitivity (deviation from the norm=normergy) in a qualitative sense. In 1964 Gell and Coombs (Gell & Coombs 1964) proposed a classification of allergic reactions in four categories (I-IV). A difficulty is that this classification of allergic mechanisms, which can be studied in a more or less isolated pure form in animal models, has been sometimes applied to human diseases in which the mechanisms are often still incompletely understood and are certainly more complex than such categorization implies (Middleton et al. 1978).

The characterisation of reaginic antibodies in serum samples from ragweed sensitive patients by Ishizaka (Ishizaka et al. 1966) and the discovery of the myeloma protein ND by Johansson (Johansson & Bennich 1967) has led to the recognition of a new class of immunoglobulins, immunoglobulin E or IgE. In this thesis the term 'allergy' will refer to reactions based on an interaction between allergen and specific IgE antibodies.

2.2.3 IgE mediated allergic reactions

Allergen exposure leads to a cascade of immunological events in IgE-mediated allergy. The events are dependent on the specific triggering of IgE - sensitised mast cells and basophils by antigen resulting in release of mediators of inflammation.
The clinical consequences of this cascade are symptoms of the nose such as sneezing, rhinorrhoea and nasal blockage, bronchial asthma and skin disorders such as urticaria or atopic dermatitis. The symptomatology of a patient depends on the occurrence of allergen-specific IgE and the capacity of mast cells and basophils to release mediators (releasability), but also on the degree of non-specific hyperresponsiveness of the end organs (nose, lungs, skin).

In the next three paragraphs the cells and their mediators will be discussed with respect to data obtained from in-vitro studies and investigations of the skin and lungs. The following two paragraphs are focus on data obtained from studies in the nose.

2.2.3.1 Mast cells, basophils and their mediators

Cells primarily involved in the allergic reaction include mast cells and basophils. Two distinct categories of mast cells have been demonstrated in rodents: mucosal mast cells (MMC) in small-intestinal mucosa and connective tissue mast cells (CMTC) in small intestinal submucosa, skin, skeletal muscle and serosal surfaces. Heterogeneity of human mast cells has been described (van Overveld 1988, Irani & Schwartz 1989). Human mast cells can be characterised by the presence of tryptase on the one hand (T mast cells) or tryptase and chymase (TC mast cells) on the other (Irani & Schwartz 1989). Another classification has been based on the sensitivity of the cells to formalin with respect to staining with alcian blue (van Overveld 1988). In terms of tissue localisation human T mast cells correspond most closely to rodent MMC, while human TC mast cells correspond to rodent CTMC (Irani & Schwartz 1989). Formalin sensitive mast cells are comparable with T mast cells, whereas formalin insensitive mast cells correspond with TC mast cells (van Overveld 1989). The two mast cell types display a different biochemical composition. For instance T mast cells release larger amounts of leucotriene C_4 (LTC_4) than TC mast cells do. Heterogeneity of mast cells will be responsible for heterogeneity of mediator release on stimulation. Accordingly, mast cells from the skin

(predominantly TC) release histamine in response to a variety of substances such as compound 48/80, morphine sulphate, polylysine, f-met peptide and C5a, whereas lung mast cells (predominantly T) will not respond to these stimuli (Irani & Schwartz 1989).

Binding of allergen to specific IgE-molecules on mast cells and basophils can be considered a starting point of a series of events finally resulting in allergic symptoms. A major characteristic of IgE is the ability to bind to the high affinity IgE receptors (Fc_eI) on mast cells (Tomioka & Ishizaka 1978) and basophils (Ishizaka et al. 1966). Once IgE is bound to the Fc_eI receptors, degranulation may be triggered by crosslinking or bridging of two adjacent IgE molecules by allergens (Chabai et al. 1980, Ishizaka et al. 1970). Calcium influx induced by this bridging or by other mechanisms will lead to the release of mediators. These mediators are stored preformed in secretory granules (primary), or synthesized de novo on cell activation (newly generated). By the action of some of these mediators, other cells such as eosinophils release (secondary) mediators (Schwartz & Austen 1988). Major primary mediators produced by mast cells are histamine, proteoglycans such as heparin and chondroitin sulphate, neutral proteases tryptase and chymase and acid hydrolases (Schwartz & Austen 1988). Newly generated mediators of mast cells include adenosine, oxidative metabolites of arachidonic acid, such as prostaglandin D_2 (PGD_2), leucotriene C_4 (LTC_4), and leucotriene B_4 (LTB_2) (Lai & Holgate 1988). Synthesis of platelet activating factor (PAF) in human lung mast cells has been described but none of this PAF-acether could be found in the extracellular environment (Schleimer et al. 1986). Human basophils generate LTC_4 but no prostaglandin products nor PAF (Irani & Schwartz 1989, Schwartz and Austen 1988). Secondarily derived mediators from cell sources other than mast cells or basophils may include additional prostaglandins, leukotrienes, chemotactic factors, kinins, anaphylatoxins (Schwartz and Austen 1988) and PAF (Dahl et al. 1985).

2.2.3.2. Other cells

Cells other than mast cells and basophils are involved in the allergic reaction. IgE receptors are not exclusively restricted to mast cells and basophils. Low affinity Fc_eII receptors on B- and T lymphocytes may have a regulatory role in B- cell growth and IgE-synthesis (Conrad 1989). Allergen, through IgE fixed to low-affinity Fc_eII receptors

can activate eosinophils, monocytes and platelets (Capron et al. 1986).
Recently the binding of IgE to Langerhans cells was demonstrated (Bruijnzeel-Koomen 1989, Fokkens et al. 1989a, Fokkens et al. 1989b). IgE on Langerhans cells may be important in antigen presentation as it has been demonstrated that in atopic dermatitis IgE bearing Langerhans cells are required to induce a lymphoproliferative response (Bruijnzeel-Koomen 1989). The exact role of these Langerhans cells in respiratory allergy (Fokkens et al. 1989a, Fokkens et al. 1989b) remains to be established.

2.2.3.3. Inflammation and late phase reactions: the link to hyperreactivity ?
IgE mediated allergy does not imply symptoms only occurring immediately after allergen exposure. Blackley already noted symptoms of asthma several hours after allergen inhalation that lasted several days (Blackley 1873). Herxheimer observed two distinct components in the response to inhaled allergen, an immediate asthmatic attack which he called the immediate and and a late reaction mostly 7 to 10 hours later (Herxheimer 1952). Both Booij-Noord (Booij-Noord et al. 1972) and Pepys (1973) demonstrated a dual response of the lower airways to inhalation of house dust extract. It is now clearly recognized that the IgE-mediated allergic response may be characterised by two distict phases, an early phase developing within 10 minutes after allergen exposure and a late phase occurring 3 to 4 h later with a maximum at 6 to 8 hours after allergen exposure (Gleich 1982, O'Byrne et al. 1987).
Several studies point to a link between allergic reaction and hyperreactivity. The observation that grass pollen exposure during the grass pollen season could increase bronchial hyperresponsiveness in sensitized persons was made by Altounyan (Altounyan 1970). Subsequently Cockroft and coworkers (Cockcroft et al. 1977) demonstrated that the increase in airway responsiveness to histamine or methacholine only occurred in patients with a late phase reaction. Cartier et al. (1982) demonstrated that the magnitude of the late phase reaction is significantly correlated with the increase of bronchial hyperresponsiveness. Recently it was shown that increase in airway responsiveness to histamine precedes the start of a late phase reaction in asthmatic patients (Durham et al. 1988).
It has been hypothesized that in allergic asthma allergen exposure leads to an immediate and a late phase reaction. Bronchial hyperresponsiveness associated with the late phase

will lead to the daily symptoms of asthmatic patients. Moreover hyperresponsiveness will enhance the response on repeated allergen exposure. In this way, allergic reaction and hyperreactivity interact with each other in a vicious circle (Cockroft 1983).

Apart from bronchial asthma late phase reactions have also been demonstrated in the skin (Solley 1976, Gleich 1982) and the nose (Pelikan 1978, Dvoracek et al. 1984, Naclerio et al. 1985).
When surveying the literature it appears that a variety of inflammatory cells such as mast cells, basophils, neutrophils, eosinophils, macrophages and platelets have been implicated in the allergic reaction (Kay et al. 1988). After allergen challenge in asthmatic patients an increase of eosinophils in broncho-alveolar lavage (BAL) has been demonstrated (de Monchy et al. 1985). Another study showed an increase both in neutrophils and eosinophils (Metzger et al. 1987b). The two studies differed in the time at which lavage samples were taken. A study performed by Kirby (Kirby et al. 1987) supports the role of inflammatory cells in bronchial hyperresponsiveness. He showed a close correlation between mast cell numbers in lavage fluid and airway hyperresponsiveness expressed as a PC_{20} for methacholine ($r=-0.94$). Also, eosinophil numbers were correlated with the degree of airway responsiveness ($r=-0.71$).
Besides the presence of mast cells the capacity of cells to release mediators (releasability) may be important. The releasability of mast cells or basophils will vary in individuals. An increased releasability of blood basophils has been demonstrated in case of bronchial asthma (Findly & Lichtenstein 1980, Tung & Lichtenstein 1982, Neijens et al. 1980, Gaddy & Busse 1986) . Both Neijens et al. (1984) and Gaddy et al. (1986) demonstrated an association between basophil releasability and bronchial hyperresponsiveness.

In several studies particular attention has been paid to eosinophils. Eosinophils accumulate during the late bronchial reaction (de Monchy et al. 1985). Eosinophil derived mediators such as eosinophil cationic protein (ECP) can also be demonstrated in broncho-alveolar lavage fluid during this late phase (de Monchy et al. 1985), which implies not only the presence of these cells but also their activation. It has been demonstrated that eosinophil derived mediators such as major basic protein (MBP),

eosinophil peroxidase (EPO) and ECP are toxic to respiratory epithelium (Ayars et al. 1989). Other biologically active mediators released by eosinophils are LTC_4 and PAF (Bruynzeel 1989). Accordingly, it can be hypothesized that activated eosinophils through their mediators play a part in the occurrence of bronchial hyperresponsiveness.
An important development in the research into inflammatory cells is the increasing information on T cell activation in respiratory allergy. A relative increase in CD8+ (T-suppressor) cells in BAL has been demonstrated in asthmatic patients who experienced only an early reaction after allergen challenge compared with subjects with both an early and late phase reaction (Gonzalez et al. 1987). A selective increase of CD4+ (T-helper) cells was seen in subjects who showed a late phase reaction (Metzger et al. 1987b). In the skin a strong correlation between influx of CD4+ T-cells and activated eosinophils after allergen challenge has been found. A variety of lymphokines produced by T-cells and other cells are involved in the growth, differentiation and attraction of other inflammatory cells such as basophils and eosinophils (Denburg et al. 1989). T-cell derived lymphokines are also able to enhance IgE-mediated histamine release and leukotrienes production (de Weck 1989). Thus, it is possible that these T-cells through their lymphokines intensify the allergic reaction. T-cell dependent eosinophilopoiesis could be important in the maintainance of the allergic response.

In conclusion, a variety of inflammatory cells is involved in the allergic reaction. In bronchial asthma the late phase reaction characterised by influx and activation of cells has been linked to bronchial hyperresponsiveness. The relation between bronchial hyperresponsiveness and late phase reactions expressed as alterations in lung function (i.e. decrease in FEV_1) is better established than the relationship between hyperresponsiveness and a specific inflammatory cell. Most data are circumstantial and support a time relationship but not a cause-effect relationship. These data are obtained from different studies concerning skin biopsies, broncho-alveolar lavage and in- vitro studies.

2.2.3.4 Cells and mediators in allergic rhinitis

The question remains to what extent inflammation and late phase nasal reactions plays a role in allergic rhinitis, in particular in nasal hyperreactivity.

Many studies focus upon inflammatory cells in relation to the allergic reaction of the nasal mucosa. Several studies emphasize the importance of mast cells with respect to nasal symptoms. The amount of histamine release (as a marker for the presence of mast cells) after allergen stimulation of nasal scrapings in vitro is well correlated with the nasal response on allergen challenge in vivo (Okuda et al. 1983b). During the pollen season an increase of mast cell numbers in nasal biopsies has been found (Viegas et al. 1987, Lozewick et al. 1990). There was an eightfold increase in the total number of mast cells in the mucous membrane. In addition the number of mast cells present in the surface epithelial layer changed from almost total absence in midwinter to counts of 2000-28000/mm^3 in the grass pollen season. Enerbäck could not demonstrate the increase in total mast cell numbers, but he observed a relative increase of intra-epithelial mast cells (Enerbäck et al. 1986). This altered distribution of mast cells during allergen exposure can be interpreted as shift of cells from the lamina propria to the epithelium, but alternatively a homing of precursor cells to the nasal mucosa and proliferation in the epithelium is possible (Otsuka et al. 1986). Metachromatic cells (mast cells, basophils) can be found superficially in the nasal mucosa (obtained by imprints) 5-24 hours after allergen challenge, with a correlation between the quantity of cells and symptom scores (Borres et al. 1990).

Basophils have been described in mucus (Okuda et al. 1983b) and influx of basophils into nasal lavage fluid has been found 4-11 hours after allergen challenge (Bascom et al. 1988). All these studies indicate that during allergen exposure mast cells and basophils are shifted to the superficial layers of the mucosa. Possibly this superficial location of mediator cells will further facilitate the binding of allergen to these cells with consequently more rapid release of mediators.

Studies of releasability of blood basophils from patients with allergic rhinitis are scanty. One study demonstrated an increased histamine release induced by deuterium oxide (D_2O) in a minority of allergic rhinitis patients (Tung & Lichtenstein 1982). Another study (Busse et al. 1986) showed increased responsiveness to ConA, a lectin which crosslinks adjacent IgE molecules. It is, however, not known as yet whether basophil releasability is associated with nasal hyperresponsiveness.

Importance of eosinophils in the nose has been found in several investigations. Allergen

challenge will induce an influx of eosinophils in nasal secretion (Mygind 1978). During the pollen season an increase of eosinophils can be found in blood (Varekamp & Voorhorst 1965) and nasal secretion (Pipkorn et al. 1988a). The eosinophils in nasal scrapings are correlated with the severity of symptoms during repeated allergen challenges (Pipkorn et al. 1989b) and natural exposure (Pipkorn et al. 1988a). Activation of eosinophils has been shown by the occurrence of MBP and eosinophil derived neurotoxin (EDN) (Bascom et al. 1989) and ECP (Linder et al. 1987) in nasal secretion. In addition, blood eosinophils from allergic rhinitis patients have a lower density, determined by centrifugation, during the pollen season than outside the season (Frick et al. 1989). The low density is caused by release of mediators implying cell activation. In vitro EPO and MBP causes lysis of nasal epithelial cells (Ayars et al. 1989). Thus, the occurrence and activation of eosinophils has been demonstrated during the nasal allergic reaction. Based on the in-vitro studies, it can be hypothesized that mediators derived from eosinophils damage the nasal mucosa.

The role of activated T-cells in the nose has to be elucidated. It is not known whether the relationship between activated T-cells and eosinophils observed in the skin can be demonstrated in the nose. In this respect the findings of Fokkens et al. (1989b) are of interest. She observed an increase of IgE bearing Langerhans cells during the pollen season. The idea of T-cell stimulation by antigen-presenting Langerhans cells is tempting.

Several mediators have been found in nasal secretion: histamine, prostaglandin D_2 (Naclerio et al. 1983), LTC_4 (Shaw et al. 1985), mast cell tryptase (Castells & Schwartz 1988), ECP (Linder et al. 1987), MBP (Bascom et al. 1989), eosinophil derived neurotoxin (EDN) (Bascom et al. 1989), PAF (Miaddonna et al. 1989) and kinins (Proud et al. 1983). The importance of histamine as a mediator in nasal allergy can be derived from the therapeutic effect of antihistamines. The histamine content of the nasal mucosa is correlated with the severity of nasal symptoms in the pollen season (Pipkorn et al. 1988b). However, antagonists of histamine (Wihl et al. 1985b) or inhibitors of histamine synthesis (Pipkorn et al. 1987) will not protect sensitized patients against allergens-exposure completely. In particular, antihistamines are less effective in

ameliorating nasal passage than in suppressing nasal secretion and sneezing (Wihl et al. 1985b), which suggests involvement of other mediators. A few experiments have been performed with application of other mediators. Application of LTC_4 and its metabolite D_4 in the nose leads to nasal blockage (Miadonna et al. 1987, Okuda et al. 1988). Platelet activating factor (PAF) has been considered as unimportant in nasal allergy, as this mediator leads to vasoconstriction of nasal vessels and decrease of nasal resistance (Pipkorn & Karlsson 1984). Moreover, PAF provocation could not induce an increased responsiveness to allergen (Andersson & Pipkorn 1988). These findings, however, do not fit in with the variety of potent biological effects of this mediator such as increase in vascular permeability, platelet and neutrophil activation and eosinophil chemotaxis and activation (Barnes et al. 1988). A preliminary study by Miadonna et al. (1989) demonstrated that PAF provocation induces an increase of nasal resistance. However, it is not known whether experiments of this kind reflect the in-vivo events during the allergic reaction. Whenever specific antagonists of leukotrienes and PAF become available, the role of these mediators in allergic rhinitis may be elucidated.
In conclusion, the occurrence of inflammatory cells such as mast cells, basophils, eosinophils and their mediators has been demonstrated during the nasal allergic reaction. Although some mediators such as histamine and leukotrienes may be biologically active in allergic rhinitis, the role of other mediators such as tryptase, chymase, PAF etc. needs further clarification.

2.2.3.5 The relation between IgE-mediated allergy and hyperreactivity in the nose

One of the first reports on the relation between hyperreactivity and allergy was by van Lier and van Dishoeck in 1960 (van Lier 1960, van Dishoeck & van Lier 1960) who showed that during the grass pollen season patients with grass pollen allergy demonstrated an increase in nasal sensitivity to intranasally applied veratrine, a mixture of alkaloids.
In the late sixties the increased sensitivity to allergen was studied intensively by Connell (Connell 1968a), who showed that repeated exposure to ragweed pollen in patients with a ragweed pollen allergy increased the sensitivity to ragweed pollen. This phenomenon which he called the priming effect was local and restricted to the provoked side of the nose. Interruption of the exposure to allergen led to restoration of the normal responses

of the nasal mucosa. Priming of the nose by ragweed pollen induced an increase in sensitivity to other allergens. Biopsies of the mucosa after such repeated exposure showed eosinophil infiltration and basement membrane changes (Connel 1968b). The basement membrane changes could not be confirmed by other studies (Fokkens et al. 1990, Lozewick et al. 1990). Connell attributed the priming effect to an increased permeability of the mucous membrane, which allows the allergen to penetrate more readily to the target cells.

Borum (Borum et al. 1983) demonstrated an association between allergen exposure and nasal hyperreactivity. In grass pollen allergic patients during the pollen season nasal sensitivity increased not only to allergen, but also to non-specific stimuli such as histamine and methacholine.

Several investigators failed in priming the nose with non-immunological stimuli. It was not possible to prime the nose to ragweed pollen by means of exposure to NH_3 (Bacon et al. 1981). Grønberg was unable to enhance nasal sensitivity to histamine by repeated challenge with histamine or methacholine (Grønberg et al. 1983). In contrast, allergen provocation induced an increase in nasal sensitivity to histamine (Linder et al. 1987, Andersson et al. 1989) and methacholine (Klementsson et al. 1990). The recent observation that the increase of nasal sensitivity to histamine was very well correlated with increase of sensitivity to allergen (Andersson et al. 1989) suggested a common pathogenesis.

Research into the relationship between late phase allergic reactions in the nose and non-specific hyperreactivity has been hampered by the lack of well- defined methods to determine late phase reactions in the nose. Although late phase reactions have been observed by measurement of the nasal airway resistance (NAR) by Pelikan (1978), Taylor et al. (1971) and Dvoracek et al. (1984), Richardson was unable to demonstrate the occurrence of such a phenomenon (Richardson & Seebohm 1979). It has been postulated that the baseline variation in NAR (Kumlien & Schiratzki 1979) and the presence of a nasal cycle (Eccles 1982) make determination of a late phase response impracticable. Recently Mygind failed to demonstrate a clinical late phase response (recurrence of nasal obstruction, sneezing or rhinorrhoea) after allergen challenge (Mygind et al. 1988).

Determination of the early and late phases has been made possible by the development

of more sophisticated methods using mediators in nasal lavage fluid as markers of an allergic response after nasal challenge. In 1985 Naclerio et al. characterised the late phase response by a recurrence of biochemical mediators (histamine, tosyl-l-arginine methyl ester [TAME]-esterase and kinin) in nasal lavage fluid 3-11 hours after nasal challenge with ragweed pollen. The absence of prostaglandin D_2 (a mediator released by mast cells but not by basophils) suggests an involvement of basophils in the late phase response. Recently an influx of both basophils and eosinophils could be demonstrated during the late phase (Bascom et al. 1988).

In summary, various studies demonstrate the occurrence of inflammation in nasal allergy just as in bronchial asthma. Other studies focused on nasal priming and allergen-induced hyperreactivity. It can be hypothesized that allergen exposure leads to a late phase reaction and inflammation with consequent priming or increased hyperreactivity. However, the exact mechanism of hyperreactivity or nasal priming is not clear. A redistribution of mast cells might explain nasal priming but not the closely related nasal hyperresponsiveness to histamine. Inflammatory cells such as eosinophils could release mediators with toxic effects on the nasal mucosa, thereby causing nasal hyperreactivity. The evidence for this hypothesis is circumstantial. The occurrence of certain cell populations together with the induction of nasal hyperreactivity after allergen challenge implies a time relationship. In how far this relationship is also a causal one has yet to be demonstrated. Very few investigations have been designed to correlate mediator levels with non-specific hyperreactivity. In one study only a weak correlation between ECP and allergen-induced hyperreactivity to histamine could be established (Linder et al. 1987). A second study (Klementsson et al. 1990) could not confirm the relationship between eosinophil influx into nasal lavage fluid and nasal hyperreactivity. Moreover, the association between a late phase reaction and allergen-induced hyperreactivity, which is considered to be of such importance in bronchial asthma, remains to be established in the nose.

Thus, the connection between inflammation and hyperreactivity is even less well established in allergic rhinitis than in allergic asthma. Partly, the previously discussed difficulties assessing nasal hyperreactivity may be responsible for this. A first step to connect inflammation and nasal hyperreactivity might be the research into late phase

reactions in relation to allergen-induced nasal hyperreactivity.

The knowledge of the allergic reaction, of the complex immunological interactions during these reactions and of the conditions required to induce an IgE mediated response have exceeded the available information on nasal hyperreactivity. This knowledge, however, is derived from a variety of studies: in-vitro studies, animal experiments, studies with skin and nasal biopsies, nasal and bronchial lavages. Consequently, the pathophysiological mechanisms which are considered important in allergic reactions lack a solid basis of consistent data.
Nasal hyperreactivity may be the main consequence of this complex cascade in patients with an allergic rhinitis. Therefore, assesment of nasal hyperreactivity may provide an essential link between clinical symptomatology characteristics of these patients and the complex cascade of immunological events.

2.3. Classification of rhinitis

The term 'rhinitis' refers to an inflammatory process in the nose. Inflammation is characteristic of infection or allergy, but some forms of nasal disease such as 'vasomotor rhinitis' are not characterized by an inflammatory process. Therefore the general term 'rhinopathy' would be more appropiate to cover the symptomatology of sneezing, rhinorrhoea or nasal blockage irrespective of the cause. Rhinopathy should not include causes of nasal disease such as anatomical disorders, foreign bodies, specific infections, pregnancy, auto-immune diseases, and malignancies. Nevertheless, the term 'rhinitis' is commonly used in the international literature.
When surveying the literature, it appears that no uniformly accepted system is used for the definition and classification of rhinitis. Classifications have been made according to the presence of acute or chronic symptoms (Becker et al. 1989), the presence of an immunological mechanism (Connell 1983), or the occurrence of inflammation (Meltzer et al. 1989). In the classification of Dieges and Wentges (1979) the term 'rhinopathy' is used. They distinguished six categories of nasal disease: atopic rhinopathy, non-atopic rhinopathy, rhinopathia vasomotoria, infectious rhinitis, polyposis nasi and rhinitis medicamentosa. Patients with atopic rhinopathy are characterised by symptoms of sneezing, rhinorrhoea and nasal obstruction due to the interaction between IgE-antibodies

and allergens. Patients with non-atopic rhinopathy may have perennial symptoms of sneezing, rhinorrhoea and nasal obstruction without presence of an IgE-mediated allergy. Vasomotor rhinitis is characterised by attacks of frequent sneezing and a pronounced watery rhinorrhoea. This disease often occurs in women. Between the attacks the patients are free of symptoms. The authors state that the distinction between non-atopic rhinopathy and rhinopathia vasomotoria is often difficult to make.
Infectious rhinitis often begins with a viral infection. After a few days purulent nasal discharge is seen, caused by bacterial superinfection. Nasal polyps (polyposis nasi) are seen more frequently in non-atopic subjects than in patients with atopic rhinopathy. Some patients show the triad of polyposis nasi, non-atopic asthma and intolerance to aspirin and other non-steroid anti-inflammatory drugs (NSAIDs). The nasal congestion in rhinitis medicamentosa is often caused by abuse of decongestant nose drops.

The patients examined in the following series of studies belong to the subgroups of allergic rhinitis, characterised by IgE-mediated allergy, perennial non-allergic rhinitis (similar symptoms as in allergic rhinitis, but without skin reactions to inhalant allergens or bacterial infections), and infectious rhinitis, characterised by chronic or recurrent bacterial infections. Patients with the classical features of vasomotor rhinitis (attacks of profuse rhinorrhoea and sneezing, predominantly in women) form a subgroup of the perennial non-allergic rhinitis patients. Instead of rhinopathy the term 'rhinitis' is used in accordance with the international literature.

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PART II. MEASUREMENT OF NASAL HYPERREACTIVITY

3 Influence of the delivery system on the nasal mucosa

4 A comparison of nasal responsiveness to histamine, methacholine and phentolamine in alergic rhinitis patients and controls.

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Clin Allergy 1987;17:563-70.

5 Nasal hyper-responsiveness to histamine, methahcoline and phentolamine in patients with perennial non-allergic rhinitis and in patients with infectious rhinitis.

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Clin Otolaryngol 1990, in press

CHAPTER 3. Influence of the delivery system on the nasal mucosa

INTRODUCTION

In several studies it has been demonstrated, that nasal challenge with saline may induce a nasal response. Both McLean et al. (1976) and Corrado et al. (1986) observed a significant increase in nasal airway resistance after challenge with saline.

In the first two studies in this series (covered in chapters 7 and 9) challenge solutions were administered using a DeVilbiss atomizer connected to a pressure pump according to a previous protocol (Sanwikarya et al. 1985). In these studies 0.2 ml of challenge solution was sprayed into each nostril. The pressure pump delivered an airflow of 9 l/min. As a strong airstream was generated through the small aperture of the De Vilbiss atomizer, the question was raised whether the delivery system itself could induce a nasal response.

Two pilot studies were performed. The purpose of the first study (study a) was to evaluate the effect of the delivery system by challenging the noses of healthy subjects and rhinitis patients with saline. The purpose of the second study (study b) was to test the reproducibility of the output of different delivery systems.

METHODS

Study a. Thirteen patients with allergic (n=9) or non-allergic (n=4) rhinitis and ten healthy subjects underwent a provocation with phosphate buffered saline (PBS). In the case of the patients, antihistamines were withheld for two days before the test. None of the subjects used topical corticosteroids or long-lasting antihistamines such as astemizole. Airway infections during the two weeks preceding the tests had been excluded.

On each occasion subjects waited half an hour before the test to allow the nasal mucosa to become acclimatised. After rhinoscopy 0.2 ml of a solution of saline was sprayed into the nostrils by use of a De Vilbiss atomizer connected to a pressure pump. After 10 minutes the nasal airway resistance of each nostril was measured using a passive anterior rhinomanometer (Heyer PAR) (Clement et al. 1978). This entailed blowing an airstream with a constant flow of 0.25 l/sec into each nostril. The resistances for the left (R_l) and the right (R_r) cavities were calculated by dividing the nasal pressure by the flow. The total nasal resistance was computed from the equation: $R_{tot}=R_l x R_r/(R_l+R_r)$.

Study b. Four delivery systems were tested. Three nasal pump sprays (A, B, C) obtained from different manufacturers were filled with water. Ten times the output of each delivery system was measured by weighing the content of each dose each time. In addition the output of the DeVilbiss spray (D) was measured ten times.

Statistics. To compare the effects of the delivery system in the patient and control groups the two-sided Wilcoxon rank sum test was used. In order to determine the reproducibility of the output of the delivery systems the mean, the standard deviation and the coefficient of variation were calculated for each delivery system. From the coefficient of variation (V) the standard error SE(V) was calculated (Kendall and Stuart 1977). The statistical significance of a difference between two coefficients of variation was tested by calculation of the t-value and the corresponding degrees of freedom. A p-value of $<= 0.05$ was considered statistically significant.

RESULTS

Study a. The NAR after provocation with saline was expressed as percentage of the baseline. Nasal challenge yielded a larger NAR in the rhinitis patients than in the control group (median: 91 vs 117 % ;p=0.0007, Wilcoxon rank sum test, figure 1). Median baseline values and ranges before challenge in the rhinitis and control groups were 18.5 (13-48) and 20 (11-51) mm H_2O/l/sec without significant difference (Wilcoxon rank sum test; $p > 0.05$).

Study b. Mean doses delivered by the devices A-D, their standard deviation and coefficient of variaton are shown in table 1. Low coefficients of variation were obtained using pumpspray C and the DeVilbiss atomizer D. Pumpspray C had a significantly lower coefficient of variation than pumpspray A ($0.05 > p > 0.02$).

DISCUSSION

The experiments show that the delivery system used in the first two studies can elicit a nasal response.

The response to the saline solution delivered by the DeVilbiss atomizer might be considered as a reaction to non-specific mechanical stimulation, thereby perhaps

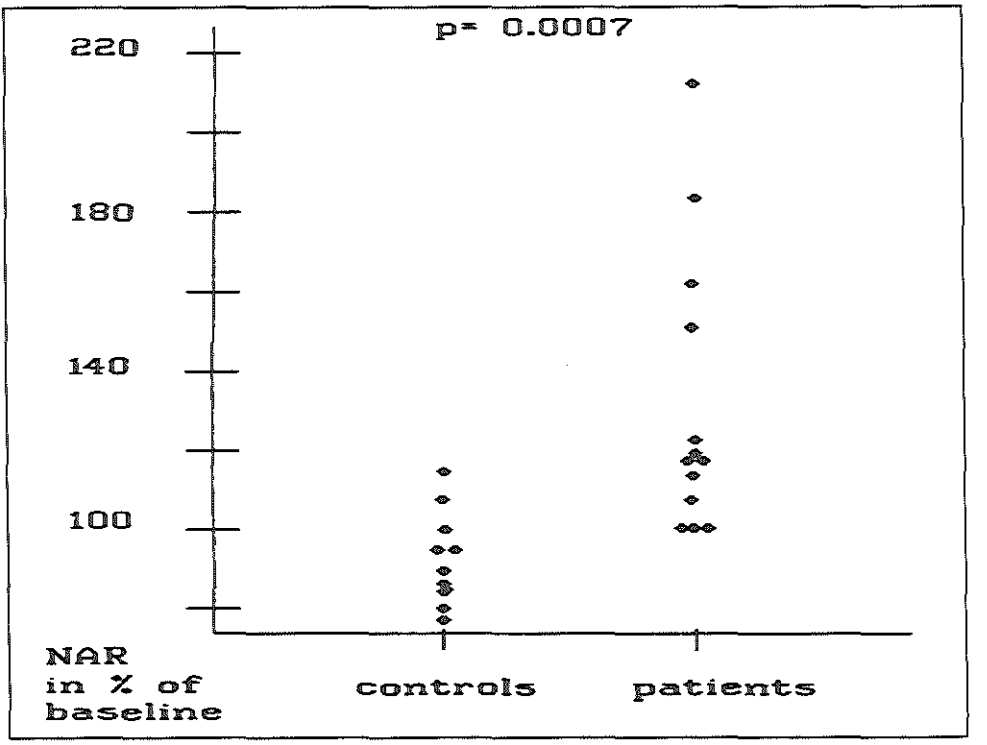

fig.1 Increase in NAR expressed in % of baseline after challenge with saline using a DeVilbiss atomizer connected with a pressure pump.

providing us with a method to characterise nasal hyperreactivity whenever the mechanical stimulation can be standardised.

Table 1. Mean of 10 measurements, sd, coefficient of variation (V) and standard error of V

Device	mean	SD	V	SE(V)
Pumpspray A	0.38	0.18	47	12.6
Pumpspray B	0.52	0.15	28	6.7
Pumpspray C	1.24	0.19	15	3.4
De Vilbiss atomizer D	1.55	0.30	19	4.4

The second study demonstrates that delivery systems may differ in the reproducibility of their output.

In order to avoid mechanical stimulation of the nasal mucosa a nasal pumpspray was

used in the studies described in chapters 4,5 6 and 8. Pumpspray C was chosen for its low coefficient of variation compared with the pumpsprays A en B. When we compare the results of this study with the investigation described in chapter 4, we cannot demonstrate any effect from a control challenge: the median NAR measured 5 minutes after saline challenge is 89 % in 11 atopic patients compared with 105% of the baseline in 17 healthy subjects ($p>0.05$; Wilcoxon rank sum test). Another session in the same study resulted in a 9 % increase in NAR after 15 minutes in 13 atopic patients vs no increase in 17 healthy subjects ($p>0.05$;Wilcoxon ranksum test).
We conclude that the results of a nasal challenge test can be biased by the mechanical stimulation of the delivery system.

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CHAPTER 4. A COMPARISON OF NASAL RESPONSIVENESS TO HISTAMINE, METHACHOLINE AND PHENTOLAMINE IN ALLERGIC RHINITIS PATIENTS AND CONTROLS.

(As published in Clin Allergy 1987;17:563-70; slightly revised).

SUMMARY

In a group of rhinitis patients with an IgE mediated allergy to house dust mites the nasal response to insufflation of histamine phosphate, methacholine and phentolamine was demonstrated to be higher than in a control group.

With the methods used histamine phosphate was better at discriminating between healthy subjects and patients than methacholine or phentolamine. This discrimination was shown by assessing the severity of reflex-mediated symptoms such as the number of sneezes and the amount of secretion and not by differences in nasal airway resistance.

INTRODUCTION

Non-specific hyperreactivity is a well-known phenomenon in bronchial asthma. Hyperreactivity is characterised by quantitative changes in the response of lung function to bronchial provocation with substances such as histamine or methacholine (1-3). Although non-specific stimuli such as damp or changes of temperature can also induce nasal symptoms in rhinitis, there are no reliable tests to measure nasal hyperreactivity in an objective way (4). Non-specific reactivity of the nose can be measured by means of nasal provocation tests with agents such as histamine and methacholine (4). However, at present a standard way of assessing the nasal response after provocation is not available. In histamine provocation the increase in nasal airway resistance (NAR) is often used as a parameter of nasal response (5-7) but the number of sneezes (8) or even a 'tickling score' (9) has been used for this purpose.

Several explanations for nasal hyperreactivity have been put forward, such as increased mucosal permeability, changes in irritant receptors or reflex activity or changes in vessels and glands of the nasal mucosa (10). The tendency of rhinitis patients to suffer from nasal stuffiness might also be explained by a nasal α-adrenergic dysfunction.

The aim of this study was two-fold. Originally we tried to establish the best agent for discriminating between allergic rhinitis patients and healthy controls, using three

provocative test agents: histamine, methacholine and phentolamine. The agents were chosen because of their different mechanisms of action on nasal mucosa. Histamine has an effect on both irritant receptors, thus stimulating nerves, and on vessels, thus causing nasal congestion (10). Conversely methacholine has a direct stimulating effect on glands (10) and phentolamine causes vasodilatation (11).
Secondly, we chose phentolamine, an α-receptor blocking agent, to investigate the α-adrenergic responsiveness of the nasal mucosa.

SUBJECTS AND METHODS

Study design

Normal subjects and selected patients with perennial rhinitis and a house dust mite (HDM) allergy underwent nasal provocation tests with histamine, methacholine and phentolamine (in this sequence) on separate days. The investigation period was restricted to 1 week for each patient. The group of healthy individuals was investigated during summer 1984 and the patients in September-November, this being the period with the highest number of house dust mites in Holland (12).

Subjects

Thirteen patients (six females and seven males), with perennial rhinitis that had lasted for more than one year, took part in the study. Their ages ranged from 19 to 31 with a median of 25 years.
Selection was based upon diagnosis of HDM allergy, confirmed by intradermal skin tests and radio-allergo-sorbent tests (RAST). With skin test titration, positive reactions were found at low concentrations (1 Noon equivalent unit/ml). Specific IgE to HDM-extract was elevated (class 3 or 4). Patients who had previously received immunotherapy, were excluded from the study. Five patients had a pollen allergy and five also had an allergy to pets (without having pets in the house). Eighteen healthy students (nine females, nine males), without clinical signs and symptoms of rhinitis or asthma, participated in the study. Their ages ranged from 21 to 35 with a median of 25 years. None of the subjects had positive skin tests for a routine series of inhalant allergen extracts, neither had they specific IgE to HDM, grass pollen or cat dander, as measured with the RAST.

The study was approved by the Ethical Committee of the University Hospital and Medical Faculty, Erasmus University, Rotterdam. All participants gave their informed consent before taking part in the study.

Agents

Histamine phosphate was used in the following concentrations : 0.25, 0.5, 1, 2, and 4 mg/ml; methacholine bromide in the concentrations 8, 16, 32, and 64 mg/ml; phentolamine in the concentrations 1, 2, 4, and 8 mg/ml. The concentrations of phentolamine were chosen after consulting a cardiologist and taking into account the fact that nasal absorption of the drug is virtually complete.

Nasal provocation tests

In the case of the patients, medication was withheld for 2 days before the test. Topical corticosteroids or long-lasting antihistamines had not been used. Airway infections during the 2 weeks preceding the tests had been excluded.

On each occasion subjects waited half an hour before the test to allow the nasal mucosa to become acclimatised. After rhinoscopy a control solution (phosphate buffered saline containing human serum albumin 0.03% and benzalkonium chloride 0.05%) was sprayed into the nostrils with a nasal pump spray delivering a fixed dose of approximately 0.125 ml solution. After provocation with the control solution, increasing doses of histamine phosphate or methacholine or phentolamine were applied in both nostrils. The interval between each dose was 5 min. during the histamine challenge tests and 15 min. during the provocation with methacholine and phentolamine.

After each provocation with histamine the subject was asked to bend forward and to collect secretion in a syringe-equipped funnel, using the method introduced by Borum (13). Sneezes were counted and just before the next provocation the NAR was measured three times. The median value was taken as the nasal airway resistance. When methacholine was used, secretion only was collected as methacholine has no effect on nasal resistance (13).

In the case of phentolamine the NAR was monitored. The nasal resistance of each nostril was measured using a passive anterior rhinomanometer (Heyer PAR) as previously described (14). This entailed blowing an airstream with a fixed flow of 0.25

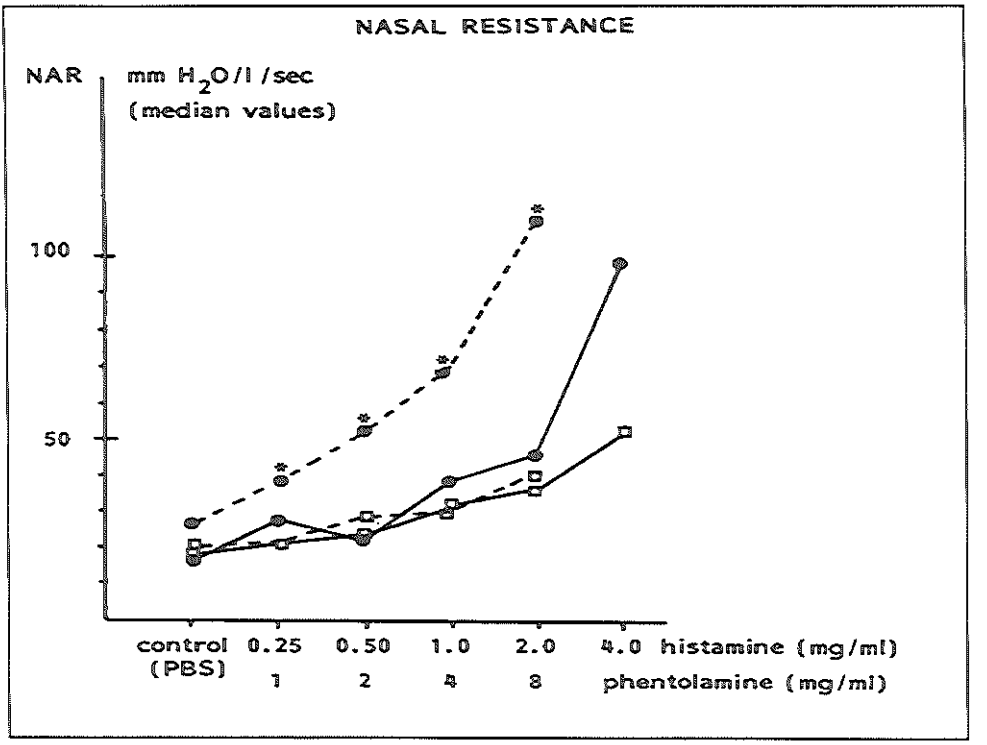

Fig. 1. Concentrations of both histamine (—) and phentolamine (- - -) and versus the median NAR for patients (●) and controls (□). *: $P<0.05$

l/sec into each nostril. The resistance for the left (R_l) and the right (R_r) cavity were calculated by dividing the nasal pressure by the flow. The total nasal resistance was computed from the equation:

$Rtot = RlxRr/(Rl+Rr)$.

Table 1. Initial NAR (*) before provocation with histamine or phentolamine

	Histamine		Phentolamine	
	controls	patients	controls	patients
median	18	19	20	27
range	12-25	11-51	11-36	14-52
significance		n.s.		n.s

(*) expressed in mm H20/l/sec.

Statistical analysis

For paired observations the Wilcoxon signed rank test was used. For comparison of the patients and controls the Wilcoxon rank sum test was used. A P value of 0.05 or less was considered statistically significant.

RESULTS

Seventeen healthy subjects participated in the histamine provocation tests and eighteen in the methacholine and phentolamine tests. In the patient group 13 histamine, 12 methacholine and 11 phentolamine provocation tests were performed. One patient was withdrawn from the phentolamine provocation test because of dizzines.

The three agents had different effects on the nose. Both histamine and phentolamine induced an increase in nasal resistance in the control and patient groups but only in the case of phentolamine was the nasal response higher in the patient group than in the control group (fig.1). No significant differences could be found in baseline NAR between patients and controls in either histamine or phentolamine provocation tests (table I). Both histamine and methacholine induced a higher secretory response in the patient group than in the control group (fig.2). In contrast, phentolamine had no effect on the nasal secretion. Histamine was capable of eliciting a sneeze reflex in the patient group (fig.3) whereas methacholine induced sneezes in only four patients and phentolamine caused no sneezes at all.

Significant side effects of the nasal provocation tests were not seen. In the case of phentolamine one patient complained of dizziness during phentolamine provocation, but there was no objective change in pulse rate and blood pressure. Phentolamine used at a concentration of 8 mg/ml caused a transient painful burning sensation in the nose, so higher concentrations could not be used.

All median values plotted in figs 1-3 represent a large range of individual values. In order to discriminate between patients and controls in a way that is easy to use in clinical practice, we used an end-point titration method. Table 2 shows the histamine end-point concentrations using three different definitions of end-point. The median end-point concentrations required to double nasal resistance do not differ between the control and patient groups. In contrast, median end-point concentration needed to give

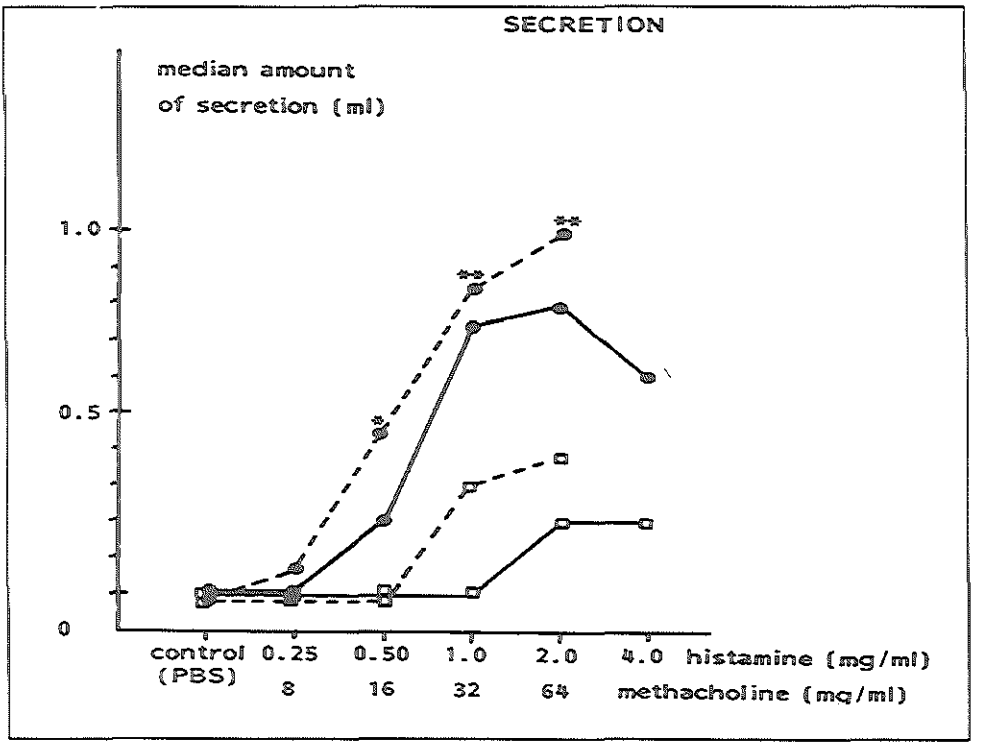

Fig. 2. Concentration of histamine (—) and methacholine (– –) and the amount of secretion for patients (●) and controls (□). *: $p<0.05$;** $p<0.01$

0.5 ml secretion and/or five sneezes is eight times lower in the patient group than in the control group (0.50 vs 4 mg/ml). A combination of symptoms does not enable a better distinction to be made between patients and controls.

A preliminary study of the variability of nasal provocation tests with histamine showed that the reproducibility of the test was better using the end-point concentrations required to produce 0.5 ml secretion and/or five sneezes (to be published).

In the case of methacholine provocation the concentration needed to produce 0.5 ml secretion was at least 5.6 times lower in the patient group than in the control group (22.6 vs 128 mg/ml or more;$P<0.02$, Wilcoxon rank sum test).

For phentolamine the concentration needed to double nasal resistance in the patient group (4 vs 8 mg/ml; $P<0.05$, Wilcoxon rank sum test) was half that required for the controls.

Table 2. End-point concentration of histamine (mg/ml) in patients and controls

Histamine end-point concentration	0.25	0.50	1.0	2.0	4.0	>4.0	
Inducing 100% increase in nasal resistance *P>0.05* (n=17)	2	1	7	1	0	6	Controls
	2	1	4	5	0	1	Patients (n=13)
Inducing at least 0.5 ml secretion and/or at least five sneezes	1	2	2	2	4 *P<0.05*	6	Controls
	3	4	3	0	0	3	Patients
Inducing 100% increase in NAR and/or 0.5 ml secretion and/or five sneezes	3	3	5	2	2 *P>0.05*	2	Controls
	4	4	4	1	0	0	Patients

DISCUSSION

Various studies of the nasal response to non-specific stimuli (5-9,13-20) have been performed, but attempts to discriminate between patients and healthy subjects have lead to conflicting results (6-8,13,15-18,20). These investigations differ from each other in the provocation technique, the way of assessing the symptoms and in selection of the patient population, which makes comparison of studies almost impossible. With our methods we observed a hyper-responsiveness to histamine, methacholine and phentolamine in allergic rhinitis patients.

Several possibilities might explain these results.

Firstly, increased permeability of the diseased mucosa may make possible a better penetration of the test agents. Conversely, the observation that histamine has the same effect on the nasal resistance of patients and controls suggests that a difference in permeability might be of minor importance. As histamine leads to exaggerated secretory response and sneeze reflex, the second hypothesis may be favoured, implying an elevated reflex-mediated activity in allergic rhinitis patients.

A third hypothesis attributes hyperreactivity to changes in glands and vessels. The tendency to hyperresponsiveness to phentolamine could possibly reflect a defect in the α-adrenergic system. Comparable assumptions have been made in bronchial asthma when using propanolol hyperresponsiveness as a measure of a defect in the β-adrenergic system. Our observation would correspond with the receptor-binding study of Ishibe et

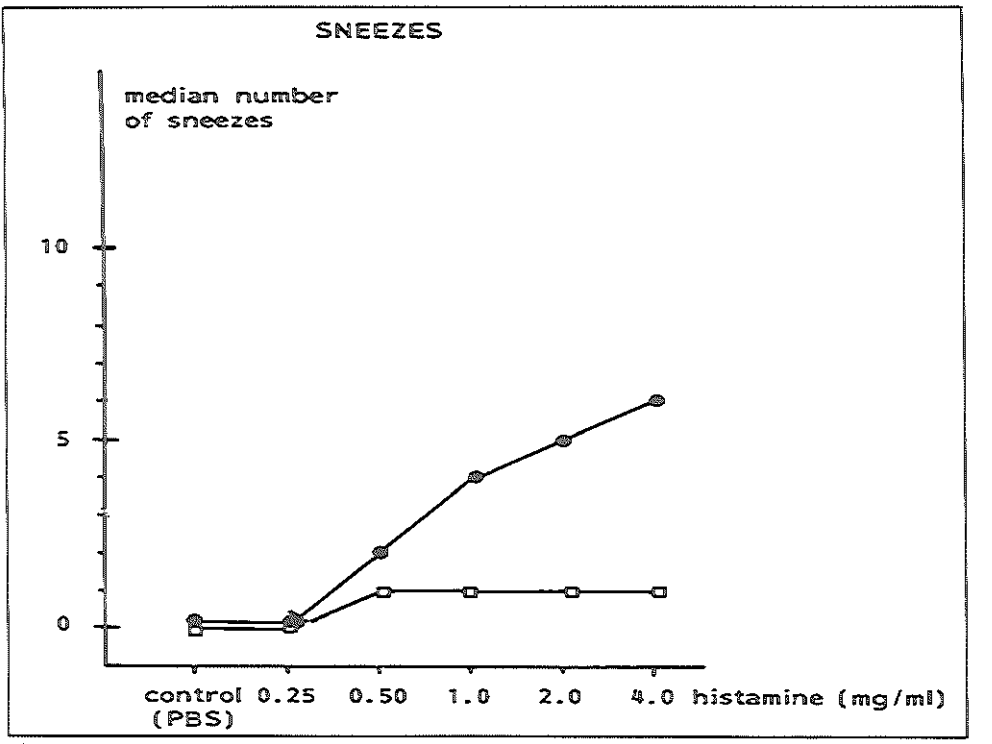

Fig. 3. Relationship between the concentration of histamine and the median number of sneezes. Controls (□), patients (●).

al (21), who showed that there was a decrease in the number of α_1 adrenergic receptors in the nasal mucosa of patients with nasal allergy. However, we cannot exclude the possibility that the response to phentolamine may merely reflect a non-specific hyperreactivity. Another problem is that there was a slight, non-significant, difference in median baseline nasal resistance between patients and controls before provocation with phentolamine. This could theoretically influence the outcome of the tests.

The finding that histamine has an equal effect on nasal resistance in patients and controls conflicts with other studies (7,8). However, in our protocol the NAR was measured after a sometimes considerable amount of secretion had been collected in a syringe-equipped funnel. Differences in nasal resistance measured after histamine provocation in other studies might be due to a difference in the production of secretion. Another possible explanation implies that measurement of total nasal resistance is less reliable in detecting differences than measurement of one-sided nasal resistance. In a recent study Corrado et

al (20) showed that only a few rhinitis patients allergic to Dermatophagoides pteronyssinus respond to histamine provocation with rhinorrhoea. In our study, however, patients were tested in autumn, as this is the season with the highest exposure to house dust mites. Recently we showed that nasal sensitivity to house dust mite and probably to histamine is increased in this season (22), perhaps due to a priming effect. The increased reflex-mediated response found in our study could reflect the active state of the disease. To our knowledge no prospective studies of nasal hyperreactivity have been carried out in large unselected patient groups. Until this is done the importance of nasal provocation tests in daily clinical practice remains uncertain.

However, by using an end-point titration method this study provides a simple way of measuring nasal responsiveness and suggests that histamine is the best agent to use in nasal provocation tests to discriminate between normal subjects and allergic rhinitis patients with active disease, provided the assessment of nasal response is focused on the reflex action of histamine (i.e. sneezes and secretion). The role of rhinomanometry in this test may be questioned. The results obtained with phentolamine provocation may reflect an α-adrenergic dysfunction of the nasal mucosa.

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CHAPTER 5. NASAL HYPERRESPONSIVENESS TO HISTAMINE, METHACHOLINE AND PHENTOLAMINE IN PATIENTS WITH PERENNIAL NON-ALLERGIC RHINITIS AND IN PATIENTS WITH INFECTIOUS RHINITIS.

(Accepted in Clin Otolaryngol)

SUMMARY.

Recently it has been shown that patients with atopic rhinitis and with an allergy to house dust mites have a stronger nasal response to insufflation of histamine, methacholine and phentolamine than a control group.

This hyperresponsiveness could not be demonstrated in patients with perennial non-allergic rhinitis, unless the patients were selected according to the predominant symptoms in the history. Patients with rhinorrhoea ('runners') proved to be hyper-responsive to methacholine compared with normal controls. The existence of two subpopulations was emphasized by hyperresponsiveness to both histamine and methacholine in the 'runners' group compared with the patients with a stuffy nose ('blockers').

Patients with chronic nasal infections (characterized by recurrent episodes of purulent discharge) showed no hyperresponsiveness at all, indicating that either hyperreactivity does not play an important part in this patient population or methods to detect hyper-reactivity in this group are inadequate. In contrast to our earlier observations in patients with atopic rhinitis increased responsiveness to phentolamine could not be detected either in the patients with perennial rhinitis or in the patients with infectious rhinitis, indicating that the possible α-adrenergic dysfunction found in patients with atopic rhinitis is restricted to this group.

INTRODUCTION

Recently nasal hyperresponsiveness to histamine, methacholine and phentolamine has been demonstrated in patients with an allergy to house dust mites (1). Determination of the histamine threshold concentration proved to be the best discriminating test for patients compared with controls, when reflex-mediated symptoms such as sneezing and secretion after provocation were used in the assessment of the nasal response (1). Although hyperreactivity is considered to be a hallmark of allergic and non-allergic rhinitis (2), it is not known whether nasal provocation tests with non-specific stimuli have the same effect in the determination of nasal hyperreactivity in different patients populations.

The aim of this study was to investigate whether other patient groups (according the classification of Mygind (3) namely patients with perennial non-allergic rhinitis or patients with chronic or recurrent infections respond to non-specific stimuli in the same manner as atopic patients do.

METHODS

Study design

Selected patients with perennial non-allergic rhinitis and patients with chronic or recurrent nasal infections underwent nasal provocation tests with histamine, methacholine and phentolamine on separate days. The investigation period was restricted to 1 week for each patient. Because of the time- consuming nature of the study several patients were unable to participate in all tests and underwent one or two provocations at random. The study was approved by the Ethics Committee of the University Hospital and Medical Faculty, Erasmus University, Rotterdam. All participants gave their informed consent before taking part in the study.

Subjects

Thirty-four patients (19 females and 15 males) with perennial rhinitis took part in the study. Their age ranged from 11 to 66 with a median of 35.5 years. They were characterized by longstanding symptoms of rhinorrhoea, sneezing and/or nasal blockage. The symptoms having been present for at least one year. Before entering the study all patients were asked about their predominant symptoms with the following choice:

rhinorrhoea and/or sneezing on the one hand or nasal obstruction on the other. Therefore they could be divided into two subpopulations: one group characterized mainly by symptoms of running nose and sneezing ('runners';n=18) and a group characterized by nasal blockage ('blockers';n=16). All patients were skin test negative to a panel of routine skin tests such as house dust mites, grass, tree and weed pollen, moulds and several pets.

Nineteen patients with recurrent or chronic infections (12 females and 7 males) also underwent nasal provocation tests. Their ages ranged from 20 to 54 with a median of 31 years. The selection of these patients was based upon their history: they all experienced episodes of purulent discharge. The diagnosis of recurrent or chronic infections was mostly made by referring ENT-specialists (15 out of 19). According to a symptom score used in the week before the test 16 patients had a period of nasal purulent discharge in this period. Three patients did not fill in their symptom score properly. None of the patients had positive skin tests for a routine series of inhalant allergenic extracts.

The patients were compared with a group of healthy students (n=18) used in the study previously described (1).

Agents

Histamine was used in the concentrations : 0.25, 0.5, 1, 2, and 4 mg/ml; methacholine in the concentrations : 8, 16, 32, and 64 mg/ml; phentolamine in the concentrations : 1, 2, 4, and 8 mg/ml.

Nasal provocation tests

All medication was withheld two days before the test and topical corticosteroids and long-lasting antihistamines were withheld 3 weeks before the test.

On each occasion subjects waited half an hour before the test to allow the nasal mucosa to become acclimatized to the test environment. After rhinoscopy a control solution (phosphate buffered saline containing human serum albumin 0.03% and benzalkonium chloride 0.05%) was sprayed into the nostrils with a nasal pump spray delivering a fixed dose of 0.125 ml solution. After provocation with the control solution increasing doses of histamine, methacholine or phentolamine were applied to both nostrils. The interval between each doses was 5 minutes in the case of histamine and 15 minutes in

the cases of methacholine and phentolamine.

After each provocation with histamine the subject was asked to bend forward and to collect secretion into a funnel, using the method introduced by Borum (4). Sneezes were counted and just before the next provocation the nasal airway resistance (NAR) was measured three times. The median value was taken as the nasal airway resistance. When methacholine was used, only secretion was collected, as methacholine has no effect on nasal resistance (4,5). In the case of phentolamine the NAR was monitored. The nasal resistance of each nostril was measured using a passive anterior rhinomanometer and a fixed flow rate of 0.25 l/sec (Heyer PAR) as previously described (6). The resistances for the left (R_l) and the right (R_r) cavity were calculated by dividing the nasal pressure by the flow. The total nasal resistance was computed using the equation :

$R_{tot}=R_l \times R_r/(R_l+R_r)$.

Table 1. Initial baseline values of nasal airway resistance (NAR) before provocation with histamine or phentolamine.

	Histamine	Phentolamine
Controls	18(12-25)	20(11-36)
'Runners'	20(10-58)	22(12-42)
'Blockers'	23(13-85)	25(15-88)
Infectious rhin.	21(8-209)	17(8-70)

Median NAR expressed in mm H2O/l/sec. Range between parentheses.

Statistical analysis

For paired observations the Wilcoxon signed rank test was used. For comparison of the patients and controls the Wilcoxon rank sum test was used. All calculations were made with a commercially available statistical package (STATA).

A p-value of $<= 0.05$ was considered to be statistically significant.

RESULTS

Nineteen patients with perennial rhinitis participated in the histamine provocation tests, 16 in the methacholine and 18 in the phentolamine tests. In the infectious rhinitis group 16 histamine, 12 methacholine and 11 phentolamine provocation tests were done.

Histamine induced an increase in nasal resistance in the perennial and infectious rhinitis

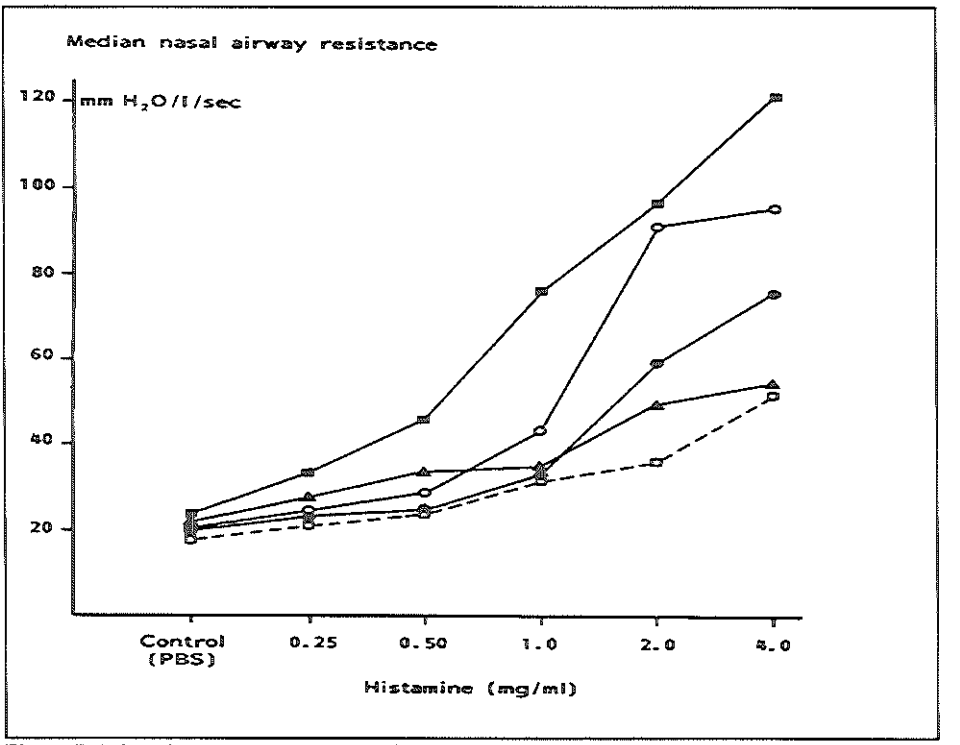

Fig. 1. Relationship between the concentrations of histamine and the median NAR in controls (□), unselected perennial rhinitis (○), 'runners' (●), 'blockers (■) and infectious rhinitis (▲).

groups, but this increase was not significantly different to the increase in nasal resistance found in the control group (fig.1). No significant differences in baseline NAR could be demonstrated between patients and controls with either histamine or phentolamine provocation tests (table 1). Both histamine and methacholine induced a secretory response in the patient groups. Again, no significant differences were seen between the patients and the controls (fig.2 ,3 and 4). When the patients with perennial rhinitis were divided into 'runners' and 'blockers' exaggerated secretory responses after histamine application with the highest doses (2 and 4 mg/ml) were demonstrated in the 'runners' (n=9) compared with the controls and with the 'blockers' (n=10) (fig.2). Histamine was capable of eliciting an exaggerated sneeze reflex in the 'runners' (fig.3). Methacholine induced a marked secretory response in the 'runners' group compared with controls, 'blockers' and infectious rhinitis patients (fig. 4).

Phentolamine caused a dose-dependent increase in NAR in all groups tested. However, median dose-response curves did not differ from the dose-response either in unselected perenial non-allergic rhinitis patients (n=18) , selected 'runners' (n=9) or 'blockers' (n=9) or in infectious rhinitis patients (n=10) compared with healthy subjects.

table 2. End-point concentrations of histamine (mg/ml) required to induce at least 0.5 ml secretion and/or 5 sneezes.

Histamine (mg/ml)	0.25	0.50	1.0	2.0	4.0	> 4.0
Controls (n=17)	1	2	2	2	4	6
'Runners' (n=9)	0	3	3	1	0	2
'Blockers' (n=10)	0	1	0	1	0	8
Infectious rhin.	1	1	1	2	1	10

Distribution of end-point concentrations. Between 'runners' and 'blockers' a significant difference was observed (Wilcoxon signed rank test; p=0.028).

As end-point titration methods are commonly used in nasal provocation tests (1,7,8,9), these methods were applied with the same definitions as previously described (1,10). The concentration of histamine required to induce at least 0.5 ml secretion and/or 5 sneezes was not distributed differently in controls compared with 'runners', 'blockers' or infectious rhinitis patients (table 2). Significant differences were seen between 'runners' and 'blockers' (p=0.028, table 2). Although the median end-point concentration was 4 times lower in the 'runners' than in the controls (1 vs 4 mg/ml), this difference was not significant. End-point concentrations required to induce 100 % increase of NAR or to induce at least one of the three symptoms (doubling of NAR and/or 0.5 ml secretion and/or 5 sneezes) did not discriminate either between the patient groups among themselves or between controls and patients. The concentration of methacholine required to elicit at least 0.5 ml secretion was significantly lower in the 'runners' compared with 'blockers' and healthy controls (p=0.014 and p=0.004 respectively, table 3). With methacholine provocation it was not possible to detect differences between 'blockers' and patients with infectious rhinitis on the one hand and healthy subjects on the other. As we expected from the dose-response curves, phentolamine hyperresponsiveness could not be demonstrated in all the patient groups.

Table 3. End-point concentrations of methacholine (mg/ml) required to induce at least 0.5 ml secretion.

Methacholine (mg/ml)	8	16	32	64	>64	
Controls (n=18)		0	0	4	1	13
						p=0.004
'Runners' (n=8)		3	1	2	1	1
						p=0.014
'Blockers' (n=8)		0	0	1	3	4
Infectious rhin. (n=12)		0	2	0	2	8

DISCUSSION

Nasal hyperresponsiveness to histamine and methacholine has been extensively studied in allergic rhinitis (1,5,8,9,11,12,13,14).

Less is known about non-allergic rhinitis. In perennial non-allergic rhinitis histamine thresholds are slightly lower than in healthy subjects (7). Borum has demonstrated that methacholine can induce an increased secretory response in perennial non-allergic rhinitis (4); however, the patient population in this study was highly selected as the patients had symptoms for 6 hr a day and used, on an avarage, 27 paper handkerchiefs a day. A second study confirmed the existence of hyperreactivity to methacholine in perennial rhinitis of non-allergic origin (13). However, in another study in patients with non-allergic chronic rhinitis patients nasal hyperresponsiveness to methacholine could not be demonstrated (14).

These discrepancies may have been due to patient selection. Perennial non-allergic rhinitis is a less well-defined disease than allergic rhinitis. The diagnosis has to be made by exclusion of specific causes (i.e. allergy and infections). Moreover patients may differ in nasal symptoms. Mygind divided patients with perennial rhinitis into 'sneezers' and 'blockers' according to their main symptoms (15). Our study shows that at least two subpopulations exist with different response patterns to histamine and methacholine. The expression 'runner' we use, corresponds to the term 'sneezer' ,as we did not differentiate between sneezing and rhinorrhoea in classifying our patients.

The end-point determinations suggest that methacholine discriminates between 'runners' and healthy subjects better than histamine does. However the validity of this observation has to be confirmed by a study with a larger number of patients, with both tests

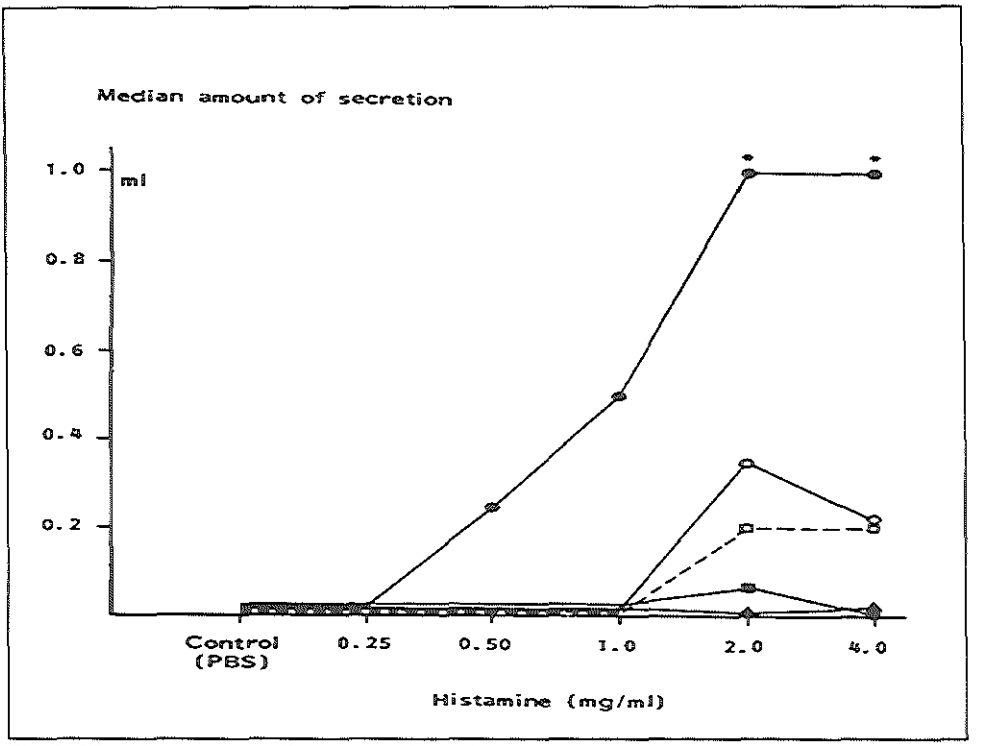

Fig. 2. Histamine and the median amount of secretion in controls (□), unselected perennial rhinitis (○), 'runners' (●), 'blockers' (■) and infectious rhinitis (▲); *:$p<0.05$.

performed in each patient. As methacholine selectively stimulates nasal glands (16,17) and histamine causes secretion both by increase of vascular permeability and reflex-mediated mechanisms (18,19) it appears that 'runners' differ from 'blockers' with respect to cholinergic stimulation and neurogenic reflexes.

To our knowledge nasal reactivity has rarely been studied in infectious disease. A temporary increase in nasal reactivity can be seen in the common cold (20).

In this study we were unable to demonstrate hyperresponsiveness to non-specific stimuli in patients with chronic and recurrent infections. These findings correspond to the observations made in bronchial asthma, as it has been established that viral infections unlike bacterial infections may increase bronchial hyperreactivity (21). A disadvantage of this study is that the selection of the patients was based on the history of recurrent or chronic infections. In 15 cases the diagnosis was established by referring ENT specialists. However, as 16 of 19 patients showed features of nasal purulent discharge in

the test period it is unlikely that the absence of nasal hyperreactivity is to be attributed to low activity or absence of nasal disease.
With respect to both 'blockers' and patients with infectious rhinitis the possibility cannot be excluded that the methods we use are insufficient to reveal hyperresponsiveness in these populations.

We have shown that measurement of NAR after histamine application offers no advantage either with respect to discrimination between patients and healthy subjects (1) or with respect to the association between test results and clinical symptoms (10). The inability to discriminate by means of NAR measurements between patients and controls in this study confirms our theory that measurement of nasal airway resistance is not required for the separation of patients and healthy subjects using histamine provocation. In contrast to our earlier observations in patients with atopic rhinitis (1) increased

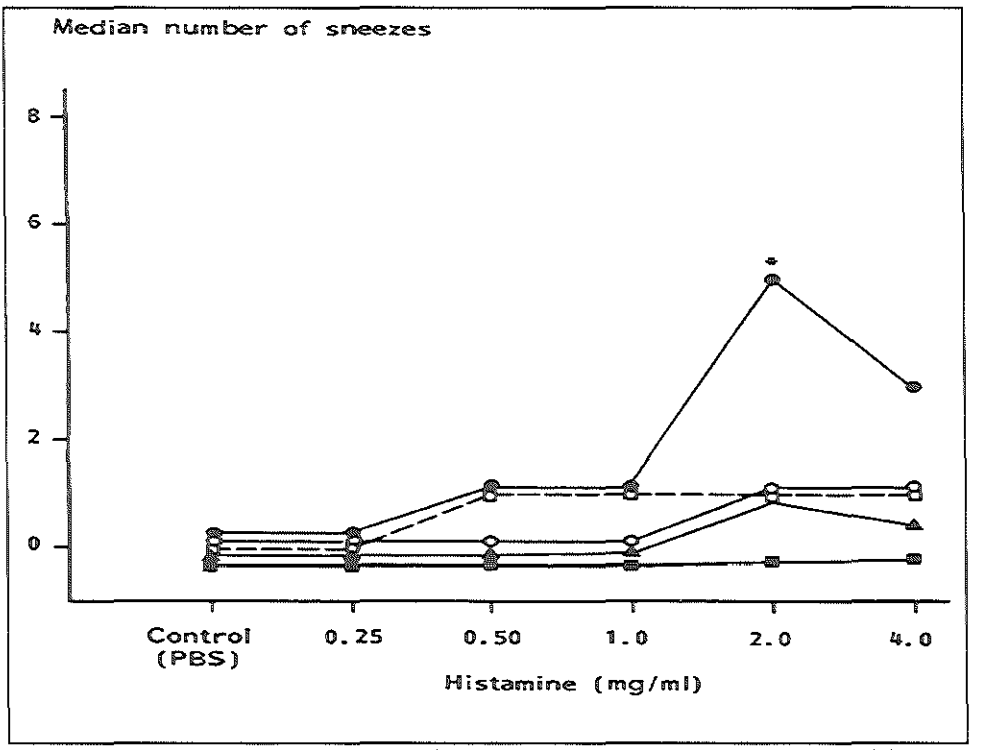

Fig. 3. Histamine versus the median number of sneezes. Controls (□), unselected perennial rhinitis (○), 'runners' (●), 'blockers' (■), infectious rhinitis (▲); *:$p<0.05$.

responsiveness to phentolamine could be detected neither in the patients with perennial rhinitis nor in those with infectious rhinitis. In our earlier study we speculated that the results could be influenced by the higher initial NAR (median: 27 mm H_2O/l/s) in the patient group compared with the healthy subjects (median: 20 mm H_2O/l/s). However, the 'blockers' in this study showed a baseline NAR of comparable magnitude (table I) without nasal hyperresponsiveness to phentolamine. Therefore, the tendency to hyperresponsiveness to phentolamine in patients with atopic rhinitis might reflect a defect in the α-adrenergic system. Comparable assumptions have been made in bronchial asthma when propanolol hyperresponsiveness was used as a measure of a defect in the β-adrenergic system (22). In nasal disease it has been established that patients with allergic rhinitis have smaller numbers of α receptors in the nasal mucosa than patients with sinusitis (23,24). Although the clinical significance of these findings is unclear it seems reasonable to suppose that a decreased density of α_1-adrenergic receptors in the nasal mucosa will facilitate vasodilatation and swelling of the nasal mucosa in nasal allergy.

We conclude that in nasal provocation studies of patients with perennial non-allergic rhinitis the existence of two subpopulations has to be postulated. So far there is no evidence that nasal hyperreactivity plays a part in recurrent or chronic infections.

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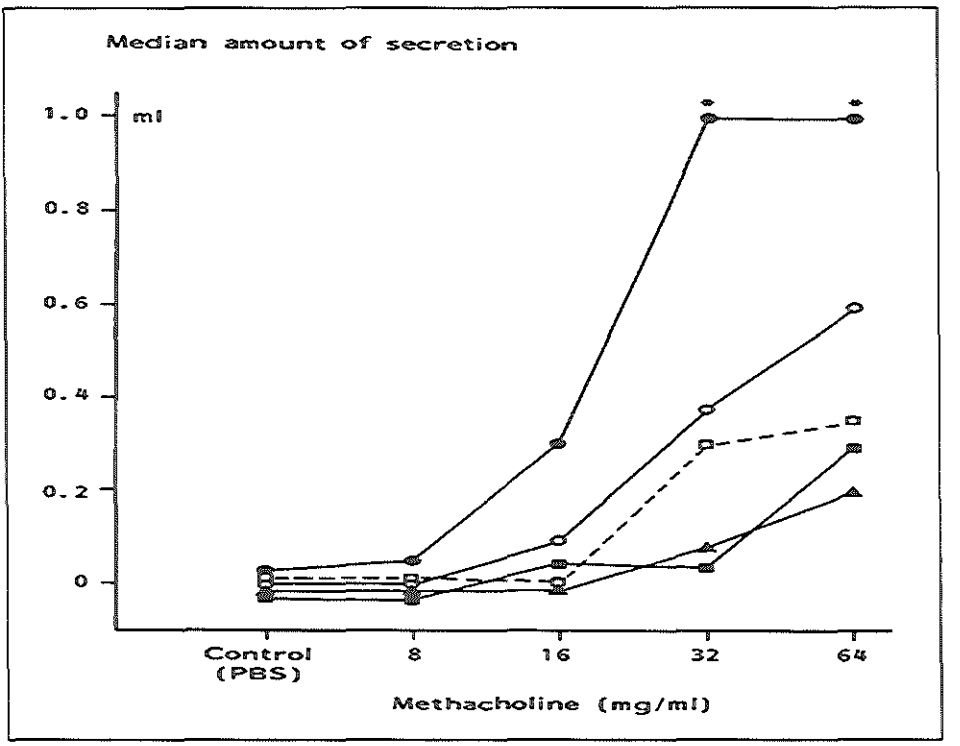

Fig. 4. Methacholine versus the amount of secretion. Controls (□), unselected perennial rhinitis (○), 'runners' (●), 'blockers' (■) and infectious rhinitis (▲).

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PART III. CLINICAL SIGNIFICANCE OF NASAL HYPERREACTIVITY IN NASAL ALLERGY

6 Nasal provocation with histamine in allergic rhinitis patients: clinical significance and reproducibility.
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Clin Exp Allergy 1989;19:293-8

7 Seasonal variability in nasal sensitivity to house dust mite extract.
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Rhinology 1987;25:41-8

8 Nasal hyperreactivity and late phase allergic reactions in grass pollen allergy.
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submitted

9 Nasal allergy to avian antigens.
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Clin Allergy 1987;17:515-21.

CHAPTER 6. NASAL PROVOCATION WITH HISTAMINE IN ALLERGIC RHINITIS PATIENTS: CLINICAL SIGNIFICANCE AND REPRODUCIBILITY.

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SUMMARY

In a group of rhinitis patients (n=12) with an IgE-mediated allergy to house dust mites, the nasal response to insufflation of histamine chloride appeared to be related to symptom scores obtained from the patients. In contrast to the sum of the nasal airway resistances (NAR) induced by all doses of histamine the total amount of secretion and total number of sneezes could be predicted from clinical scores. The reproducibility of the nasal provocation test was tested by comparison of the test results in two sessions with a one-week interval. The correlation between both sessions was highest with respect to nasal secretion ($r=0.87;P<0.001$) and the number of sneezes ($r=0.76;P=0.004$). The correlation coefficient was 0.71 ($P=0.01$), when the nasal airway resistance was used in the assesment of nasal response. A good reproducibility of the nasal provocation test was also obtained using an end-point titration method and determining the concentration required to produce 0.5 ml secretion and/or five sneezes as the end-point ($r=0.76;P=0.01$). The concentration required to double nasal airway resistance yielded a correlation coefficient of 0.56 ($P=0.052$). We conclude that the clinical significance of nasal provocation with histamine increases when, besides nasal airway resistance the amount of secretion and the number of sneezes is used in the assesment of the nasal response. Moreover, the reproducibility is better with respect to these symptoms than to nasal airway resistance. With respect to a better reproducibility the use of a summed nasal airway resistance is preferable to the end-point concentration required for a 100% NAR increase.

INTRODUCTION

In a recent study we demonstrated that allergic rhinitis patients could be distinguished from healthy controls by means of nasal provocation with histamine phosphate, provided the assessment of nasal response focuses on the reflex action of histamine (i.e. sneezes and secretion) (1). Although nasal provocation with histamine has been used in the evaluation of drugs (2-4), in basic research (5-8), and in the comparison between patients and healthy subjects (1,9-13), the relationship between clinical symptoms and the outcome of this test has not been very clearly established. Indirect evidence for a relationship between test results and symptoms has been reported by Okuda (11), Borum (14), and Konno (15) who found an increase in nasal sensitivity to histamine during the pollen season in pollinosis patients, and by a recent study describing seasonal variability in sensitivity to histamine and house dust mite extract in rhinitis patients who are allergic to house dust mites (16).

The aim of this study was to establish the relationship between symptoms and the results of histamine provocation.

A second objective was to investigate the reproducibility of this test.

SUBJECTS AND METHODS

Study design

Selected patients with a house dust mite (HDM) allergy underwent two nasal provocation tests with histamine, separated by a one-week interval. Tests were performed in the period November-April.

Information concerning the symptoms was obtained by the use of diary cards containing the symptom score system introduced by Norman (17). Patients recorded their symptoms over a two-week period, starting one week before the first provocation. Every 12 h the duration of sneezes, blockage and rhinorrhoea were recorded (0=no symptoms; 1=symptoms<30 min;2=symptoms between 30 min and 2 h;3=symptoms>2 h). The cumulative score for the six days before either provocation was used as the 'symptom score' in the statistical analysis.

In order to estimate the nasal reactivity on exposure to everyday stimuli, patients were administered a standard questionnaire listing possible causes of nasal symptoms (change of temperature, sprays: perfumes,toilet sprays etc., tobacco smoke, fog, paint smell and

cooking times). The number of provoking events were counted and used to give a 'hyperreactivity score', which therefore represents a once-measured baseline characteristic.

Subjects

Twelve patients (seven females, five males), with perennial rhinitis that had lasted for more than 1 yr , participated in the study. Their ages ranged from 19 - 43 with a median of 27 yr.

Selection was based upon diagnosis of HDM allergy, confirmed by intradermal skin tests. With skin-test titration, positive reactions were found at low concentrations (1 BU/ml ; extracts obtained from Pharmacia, Uppsala,Sweden). None of the patients had previously received immunotherapy. Patients characteristics are summarized in Table I. The study was approved by the Ethical Committee of the University Hospital and Medical Faculty, Erasmus University, Rotterdam. All participants gave their informed consent before the study.

Agents

Histamine phosphate in the concentrations 0.25, 0.5, 1, 2, and 4 mg/ml was used for the two weekly sessions.

Nasal provocation tests

Nasal provocation tests were performed as described previously (1). Medication was withheld for 2 days before the test. Topical corticosteroids and long-lasting antihistamines were withheld for 3 weeks before the test. Airway infections during the 2 weeks preceding the tests had been excluded.

On provocation day, subjects waited 30 min before the test to allow the nasal mucosa to become acclimatised. After rhinoscopy, a control solution (phosphate-buffered saline (PBS) containing human serum albumin, 0.03%, and benzalkonium chloride, 0.05%) was sprayed into the nostrils with a pump spray delivering a fixed dose of 0.125 ml solution.

Table 1. Patient characteristics

	age	clinical characteristics	IgE mediated allergies	medication
1.	43	rhinitis,asthma	house dust mites (HDM)	antihistamines, β2-sympathicomimetics
2.	31	rhinitis	HDM	topical corticosteroids
3.	30	rhinitis	gras pollen, cat epithelium, HDM	cromoglycate
4.	25	rhinitis	HDM	topical corticosteroids
5.	31	rhinitis,asthma	cat epithelium, HDM	antihistamines, β2-sympathicomimetics
6.	24	rhinitis	HDM	topical corticosteroids
7.	29	rhinitis	HDM	topical corticosteroids
8.	19	rhinitis	HDM	none
9.	21	rhinitis	HDM	topical corticosteroids
10.	28	rhinitis	HDM	antihistamines
11.	40	rhinitis,asthma	HDM	topical corticosteroids, β2-sympathicomimetics
12.	21	rhinitis	HDM	topical corticosteroids

After provocation with the control solution, increasing doses of histamine phosphate (0.25 , 0.50, 1, 2, and 4 mg/ml) were applied in both nostrils. The interval between each dose was 5 min.

After each provocation with histamine the subject was asked to bend forwards and to collect secretion in a syringe-equipped funnel during the 5 min-interval, using the method introduced by Borum (18). Secretion dripped into the funnel. Sneezes were counted and just before the next provocation the nasal airway resistance (NAR) was measured three times. The median value was taken to be the actual airway resistance. The NAR was measured using a passive anterior rhinomanometer (Heyer PAR) as previously described (19). This entailed blowing an airstream with a fixed flow of 0.25 l/sec into each nostril. The resistances for the left (R_l) and the right (R_r) cavities were calculated by dividing the nasal pressure by the flow. The total nasal resistance was computed from the equation:

$R_{tot} = R_l \times R_r / (R_l + R_r)$.

Table 2. Dose-response effects of histamine phosphate.

		NAR ($mmH_2O/l/s$)		secretion (ml)	sneezes
baseline	1st session	22	(10-38)		
	2nd session	26.5	(15-58)		
PBS	1st session	31	(15-52)	0 (0-0)	0 (0-0)
	2nd session	24.5	(16-55)	0 (0-0)	0 (0-0)
HISTAMINE (mg/ml)					
0.25	1st session	31	(13-81)	0 (0-0.7)	0 (0-5)
	2nd session	30.5	(16-60)	0 (0-0.6)	0 (0-4)
0.50	1st session	34.5	(17-90)	0 (0-1.8)	0 (0-8)
	2nd session	44.5	(31-69)	0 (0-0.6)	0 (0-6)
1.0	1st session	49	(17-140)	0.1 (0-2.0)	0.5(0-5)
	2nd session	59	(19-110)	0.15 (0-1.5)	2 (0-7)
2.0	1st session	62	(26-160)	0.16 (0-1.8)	0 (0-6)
	2nd session	72.5	(26-189)	0.33 (0-1.7)	1.5(0-7)
4.0	1st session	81	(34-258)	0.48 (0-1.6)	1 (0-9)
	2nd session	84.5	(26-258)	0.55 (0-3.0)	1.5(0-10)

Median and range (between parentheses) of NAR, amount of secretion and number of sneezes induced by PBS and histamine in both sessions. Regarding the NAR initial baseline values are shown.

Statistical analysis

In order to estimate a mean nasal response we summed the nasal airway resistances generated by the five doses of histamine (0.25-4.0 mg/ml) for each patient and each session. We also measured the total amount of secretion and counted the total number of sneezes produced in one provocation test.

The influence of a number of baseline characteristics such as hyperreactivity score, symptom score and, for NAR, its baseline measurement on the nasal response after histamine provocation, was analysed by regression of the total response on these characteristics. Calculations were made for each session and for the mean of both sessions.

Between-session stability was expressed by calculating the Spearman rank correlation between the summed NAR, total amount of secretion and total number of sneezes in both weeks, and the correlation between the end-point concentrations at which a certain nasal response was reached.

All calculations were made with a commercially available statistical package (STATA). A P-value of $<= 0.05$ (two-sided) was considered to be statistically significant.

Table 3. Summed nasal response in both sessions

	1st session		2nd session	
summed NAR ($mmH_2O/l/s$)	279	(109-575)	289	(131-623)
total amount of secretion (ml)	0.675	(0-7.0)	1.0	(0-7.0)
total number of sneezes	2.5	(0-32)	8	(0-25)

Median and range (between parentheses) of the summed nasal response generated by the five doses of histamine (concentration: 0.25-4.0 mg/ml)

Table 4. Association between nasal response and baseline characteristics

		P-VALUES		
Nasal response	explanatory variable	1st session	2nd session	mean of both sessions
summed NAR	symptom score	n.s	n.s	n.s
	hyperreactivity sc.	n.s	n.s	n.s
	both scores	n.s	n.s	n.s
	baseline NAR	n.s	n.s	n.s
	all variables	n.s	n.s	n.s
total amount of secretion	symptom score	0.013	n.s	n.s(0.072)
	hyperreactivity sc.	0.003	0.019	0.006
	both scores	0.0047	0.052	0.017
total number of sneezes	symptom score	0.037	n.s	n.s(0.087)
	hyperreactivity sc.	0.004	n.s(0.055)	0.009
	both scores	0.009	n.s	0.024

Regression analysis with the nasal response as variable to be explained and baseline characteristics as explanatory variables. The P-values referring to 'both scores" or "all variables' represent the chance that all estimated coefficients (excluding the constant) are zero; other P-values precede the sign of the estimated coefficient to which they refer. P-values close to 0.05 are shown between parentheses.

RESULTS

Table 2 shows median values and range of NAR,secretion and sneezes elicited by PBS and histamine application. Total nasal response is summarised in Table 3 for each dependent variable.

Regression analysis comparing the nasal response with baseline characteristics showed that the NAR could not be predicted from symptom and hyperreactivity scores in either session (Table 4 and Fig 1.). The combination of both these baseline scores was a significant predictor for the total amount of secretion in both session separately ($P=0.005, P=0.052$, respectively;Table 4). The mean amount of secretion over both

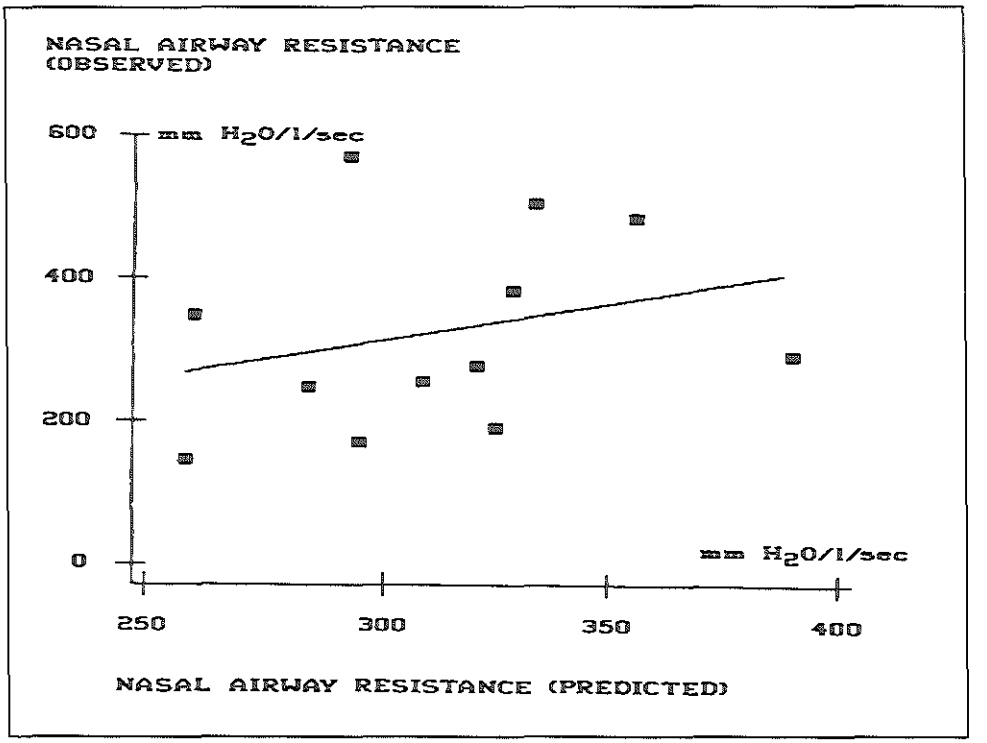

Fig. 1. Summed NAR (y-axis) plotted against summed NAR predicted from symptom score and hyperreactivity score (x-axis). The line of perfect prediction is drawn for reference.

sessions could also be predicted by both scores (P=0.017;Fig.2). The total number of sneezes could be predicted by these characteristics in the first session (P=0.009) but in the second session the effect of the combined scores was not significant (P=0.13; Table 4). The total number of sneezes over both sessions was predictable from the combination of both scores (P=0.024;Table 4 and fig. 3)
All coefficients referred to in Table 4 as significant are positive.

To investigate the relationship between nasal symptoms, we compared the summed NAR, the total amount of secretion and the total number of sneezes with each other. A high-rank correlation could be demonstrated between secretion and sneezes ($r=0.91; P<0.001$). No correlation could be found between NAR and secretion ($r=0.01; P=0.947$) or between NAR and number of sneezes ($r=-0.08; P=0.542$).
We examined the reproducibility of the nasal provocation test by comparison of the

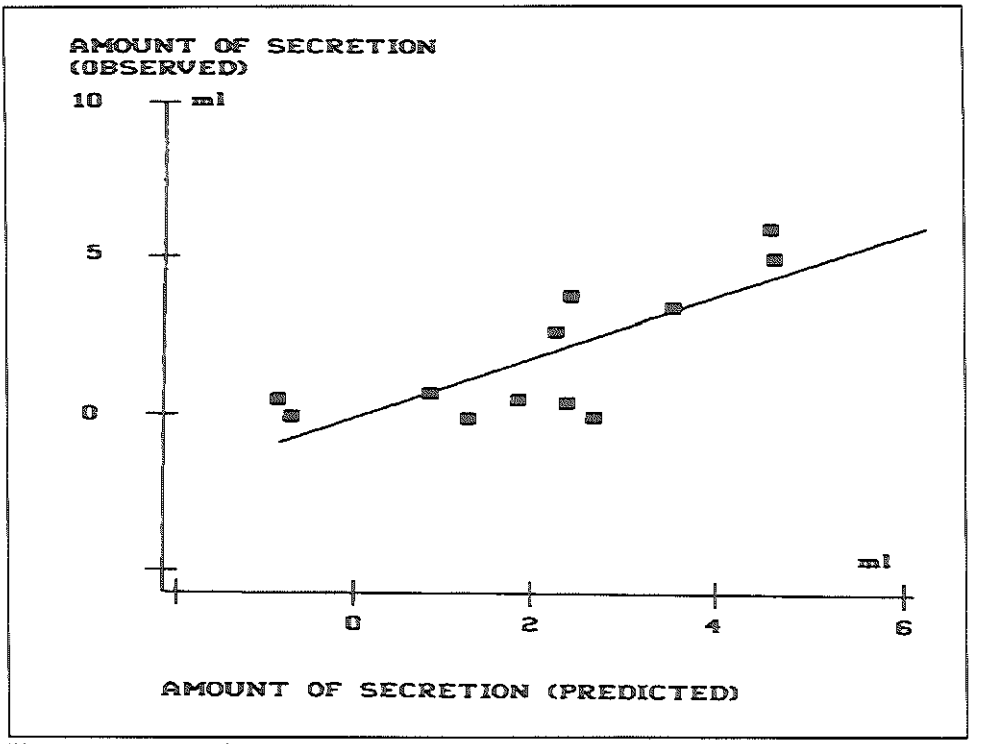

Fig.2. Total amount of secretion (y-axis) plotted against the amount of secretion predicted from the symptom score and the hyperreactivity score (x-axis).

summed NAR, the total amount of secretion and the total number of sneezes of each patient in both sessions. A good correlation between both sessions was seen when the amount of secretion or the number of sneezes was used. Spearman rank correlations were 0.87 (P=0.001) and 0.76 (P=0.004) respectively. The correlation coefficient was 0.71 (P=0.01), when the summed NAR was used in the assesment of nasal response. As end-point titration methods are commonly used in nasal provocation tests (1,10,11,13), we compared end-point concentrations in both sessions. We used the same definitions of end-point as previously described (1). The end-point concentration required to produce at least 0.5 ml secretion and/or five sneezes in the first week, was better correlated with the end-point in the second week (r=0.76;P=0.004), than either the concentration required for a 100 % NAR increase (r=0.57;P=0.052) or a combination of all symptoms (r=0.39;P=0.21).

DISCUSSION

In spite of the numerous studies of nasal histamine provocation tests (1-14), the relationship between symptoms and test results has never been very clearly established. In this study we were able to demonstrate that the results of a histamine provocation are associated with clinical symptoms. There is an assocation between nasal response (secretion and sneezes) after provocation and both the current intensity of the symptoms and the sensitivity to everyday stimuli, the latter being a hallmark of non-specific hyperreactivity (20). This assocation was not seen when the nasal airway resistance was used in the outcome of the test.

With only 12 subjects even substantial effects may easily be non-significant and this low power could be responsible for the instability of some subjects' effects, such as symptom score, on the nasal response in both sessions.

The relationship found between the reflex-mediated symptoms and clinical scores

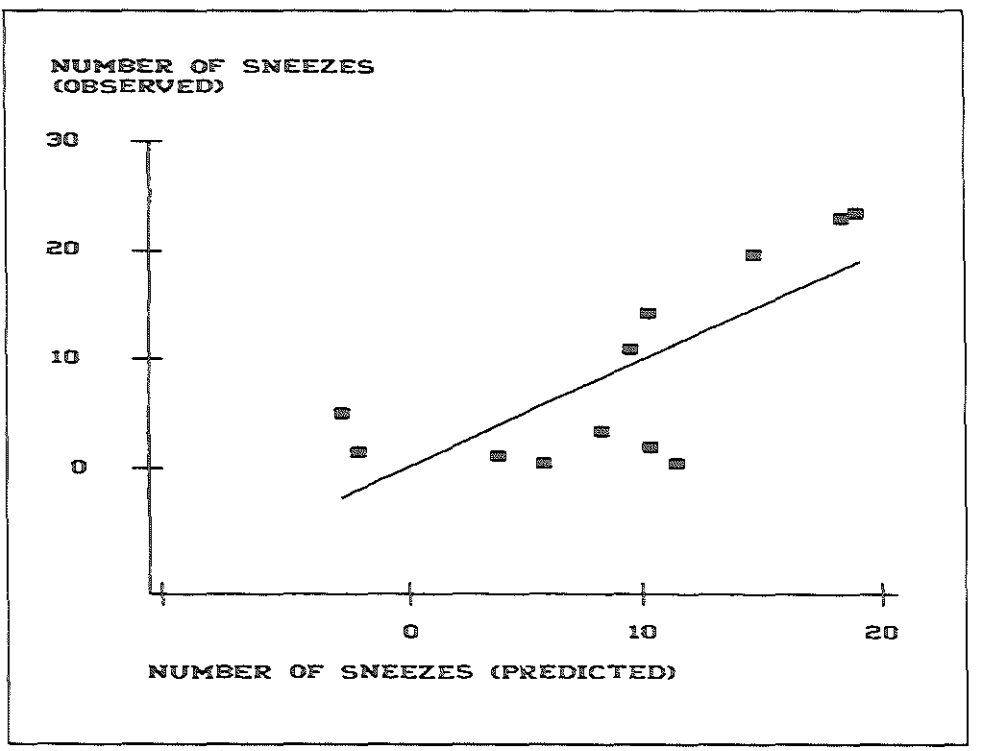

Fig. 3. Total number of sneezes plotted against the predicted number of sneezes.

corresponds with the recent observation that histamine provocation can be used to discriminate between healthy subjects and subjects with allergic rhinitis by assessing the severity of reflex-mediated symptoms but not when including nasal airway resistance in the evaluation of the test (1).

Sneezing and nasal secretion after histamine provocation are reported to be caused by stimulation of nerve endings (21) , whereas an increase in nasal resistance is the result of direct action on the nasal vasculature. The correlation between sneezes and nasal secretion in this study also indicates that their production is based on the same (reflex-mediated) mechanism.

A second objective of this study was to analyse the reproducibility of the test. We showed that the reproducibility is better when using nasal secretion or number of sneezes in the assesment of the nasal response.

In end-point titration the combination of reflex-mediated symptoms gave results that were more reproducible than those based on NAR.

The reproducibility of NAR can be increased by use of a summed NAR instead of an end-point titration method.

Borum (22) demonstrated that reasonably good reproducibility can be obtained when attention is paid to as many variables as possible including NAR. However, his study was focused upon allergen provocation, in which mediators other than histamine may play a role.

A disadvantage of simultaneously measuring NAR and nasal secretion is that vigorously clearing the nose by means of blowing or touching the nose is not possible, as these procedures will certainly effect nasal airway resistance . Therefore it is possible that the NAR we measured is not only due to congestion but is also partly caused by uncleared secretions.

The nasal response to histamine was less pronounced in the present subject group than in the subject group used in our first study (1). The main difference is that, in contrast to the present group of subjects the first group was tested in the period September-November, the season with the highest exposure to house dust mites (23). In accordance with our study of seasonal variability in nasal provocation tests (16), variability in allergen exposure can explain the difference between the studies. Secondly, the patient

groups were not matched with respect to other variables such as the degree of house dust mite allergy, sex or age.

In conclusion, the clinical significance of nasal provocation with histamine increases when reflex-mediated symptoms such as amount of secretion and number of sneezes are used in the assesment of the nasal response. Moreover, the reproducibility is better with respect to these symptoms than to nasal resistance. With respect to a better reproducibilty, the use of a summed NAR is preferable to the end-point concentration required for a 100% NAR increase.

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CHAPTER 7. SEASONAL VARIABILITY IN NASAL SENSITIVITY TO HOUSE DUST MITE EXTRACT.

(As published in Rhinology, 1987;25:41-8;slightly revised)

SUMMARY

Nine patients with a house dust mite (HDM) allergy were monitored for one and a half year in Spring 1983 during immunotherapy with aqueous alum precipitated HDM extract. Evaluation included nasal provocation tests with HDM extract and histamine phosphate. Nasal responsiveness was assessed by measurement of the nasal airway resistance, by counting the number of sneezes and measuring the amount of secretion.

During the one and a half year hyposensitization a decrease in nasal sensitivity to HDM extract is found when measurements are compared at yearly intervals (Spring 1983-1984 and Autumn 1983-1984).

However, nasal reactivity to HDM extract is elevated in autumn compared with spring (not significant in 1983, but significant in 1984).

Changes in nasal sensitivity to histamine are not as obvious except for the interval between Spring 1983 and Autumn 1983.

The fluctuations in nasal sensitivity could not be attributed to baseline variation in nasal resistance during the trial.

We conclude that seasonal variation in sensitivity to HDM can influence the results of immunotherapy with HDM extract, and should be considered when evaluating such treatment.

INTRODUCTION

In 1969 Voorhorst et al, showed that there is considerable variability in the prevalence of D.Pteronyssinus in house dust samples at different times of the year. Peak counts were observed from August until October. Studies elsewhere failed to show consistent seasonal fluctuations (Murray and Zuly, 1979), or in a study in Ohio, U.S.A., showed peak values in the warm humid months in summer (July-October)(Arlian et al., 1982). If present, a seasonal variation in the count of house dust mites (HDM) could influence the clinical evaluation of immunotherapy with HDM extract. There have been several placebo-controlled studies of immunotherapy in rhinitis patients where no mention has been made whether the time of the year has any influence on therapy (D'Souza et al., 1973; Gabriel et al., 1977; Blainey et al., 1984). Recently Pauli et al. (1984) distinguished two different periods of complaints in asthmatic patients who participated in a double-blind placebo-controlled study of immunotherapy with HDM extract. Few medications were used in the treated group until July while increased doses were given in September and October. In the placebo-treated group the mean number of medications did not change during the trial. The present paper describes a small open study of patients with clinical features of allergic rhinitis who underwent immunotherapy with HDM extract.

The results of this study suggest that there is a seasonal fluctuation in nasal sensitivity to HDM extracts and histamine.

MATERIALS AND METHODS

Patients

Thirteen patients (age range: 16-38 years) with perennial rhinitis due to HDM allergy entered the trial. Diagnosis of HDM allergy was confirmed by history, intradermal skin tests and radioallergosorbent test (RAST). No one had previously undergone immunotherapy. Other allergies (to pollen and pets) were not present.

Study protocol

The investigation period had a duration of one and a half years. Nasal provocation tests, skin tests and laboratory investigations were carried out both before the start of

immunotherapy (February-April 1983; Spring) and at half year intervals. Immunotherapy was started with weekly injections until a top dose was reached, the highest dose being repeated at two-weekly intervals.

Allergen extracts

Lyophilized HDM extract was obtained from the Diephuis Laboratory (Groningen, The Netherlands). The concentration of HDM extract was expressed in Noon Equivalent Units (NEU) as described by Voorhorst et al. (1969). The concentration used in the skin test were: 0.001, 0.01, 0.1, 1, 10 and 100 NEU/ml. For the nasal provocation tests: 1, 10, 100, 200 and 1000 NEU/ml were used. Alum precipitated HDM extract (Diephuis) was used for immunotherapy with a starting dose of 0.1 of 10 NEU/ml and a topdose of 1 ml of 10000 NEU/ml. All experiments were carried out using the same batch of lyophilized HDM extract, and care was taken to ensure that the extract remained the same strength throughout the trial by storing it at a temperature of -20 °C.

Histamine phosphate

For nasal provocation tests histamine phosphate 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml was used.

Nasal provocation tests

Medication was withheld two days before the test. None of the patients had an airway infection during the two weeks preceeding the challenge. On each occasion patients waited half an hour before the test to allow the nasal mucosa to become acclimatized. After rhinoscopy 0.15 ml of solution was sprayed into each nostril with a De Vilbiss atomizer connected to a pressure pump. In the morning provocation with increasing doses of histamine phosphate was performed at 10 minutes intervals. In the afternoon subjects were challenged with DHM extract at 15 minutes intervals. Two patients had the HDM provocation tests the morning after the histamine challenge throughout the entire study. The results with these patients did not differ from the results obtained with the other patients.

Before histamine or HDM was applied, a control solution (PBS containing HSA 0.03% and benzalkonium chloride 0.05%) was used.

The nasal resistance of each nostril was measured using a passive anterior rhinomanometer as previously described by Clement et al., 1978. This entailed blowing an airstream with a fixed flow at 0.25 l/sec into each nostril. The anterior nasal pressure of each side was measured. The resistance for the left (Rl) and the right (Rr) cavity was calculated by dividing the nasal pressure by the nasal flow. The total nasal resistance was computed from the formula: Rtot = Rl x Rr / (Rl + Rr).
The lowest concentration which doubled the total nasal resistance compared with the initial value, was taken as the end-point. As some patients reacted with secretion or sneezing instead of nasal blockage an arbitrarily chosen amount of secretion of 0.5 ml or more (collected as described before by Borum, 1978), or a total of at least 5 sneezes within 15 minutes was also taken as the end-point in these patients.

Skin tests

Intradermal skin tests were performed by injecting 0.02 ml of increasing concentrations of HDM extract. Skin reactions were read after 20 minutes and expressed using standardized plus signs following the grading system devised by Norman (1980). The plus signs were added up for each patient (Voorhorst et al., 1969; Dieges, 1983) in order to evaluate the changes in skin reactivity during the course of the trial.

Total IgE and Radioallergosorbent Test (RAST)

Total IgE was determined by a noncompetetive binding assay (Stallman and Aalberse, 1977). Specific IgE was determined by RAST (Radioallergosorbent Test) using agarose beads as an allergen-support (Adkinson, 1980) as previously described (Van Toorenenbergen et al., 1981).
The relative amounts of HDM-specific IgE were calculated from the horizontal distance between patient serum and the reference serum dilution curves, as described by Adkinson (1980).

Statistical analysis

For paired observations the Wilcoxon non-parametric signed rank test was used. Correlations were calculated using the Spearman rank correlation test. The end-point concentration in the histamine provocation test was expressed as a power of two. End-

point concentration in the allergen provocation test was expressed as a power of ten (i.e. as the logarithm).

RESULTS

Four of the thirteen patients were withdrawn from the trial.

Two stopped because of the time consuming character of the investigations, one because of large local reactions caused by the injections and one because of severe nasal blockage, which made nasal provocation tests impossible.

Analysis of HDM end-point concentrations during nasal provocations showed a significant increase in values at one year intervals (Spring 1983-1984 and Autumn 1983-1984; Figure 1) However, median end-point concentrations were lower in autumn than in spring of the corresponding year ($p > 0.05$in 1983; $p < 0.05$ in 1984). Moreover no significant ($p > 0.05$) difference in end-point values between the beginning of the trial (Spring 1983) and the end of the study (Autumn 1984) was observed.

Table 1. Initial nasal resistance before HDM provocation.

	spring 1983	autumn 1983	spring 1984	autumn 1984
Initial resistance*				
median	1.6	1.9	1.7	1.6
range	1.5-2.0	1.2-2.0	1.2-2.1	0.6-1.9
significance	n.s	n.s	n.s	

* expressed in cm H_2O/l/sec
n.s. = not significant

All patients but one had a lower nasal-end-point concentration for histamine in Autumn 1983 than in Spring 1983 ($p = 0.01$; Figure 2).

Statistically significant fluctuations were absent for the other periods measured. In all test periods significant difference in median nasal resistance could be found before provocation (Table I; $p > 0.05$).

Analysis of skin tests and RAST results for the trial periods shows a small significant decrease in median of total plus signs and a small significant increase in median RAST values without a seasonal fluctuation (Tables 2 and 3).

No significant correlation was observed between nasal threshold values and total plus signs or RAST results during the test periods ($p > 0.05$).

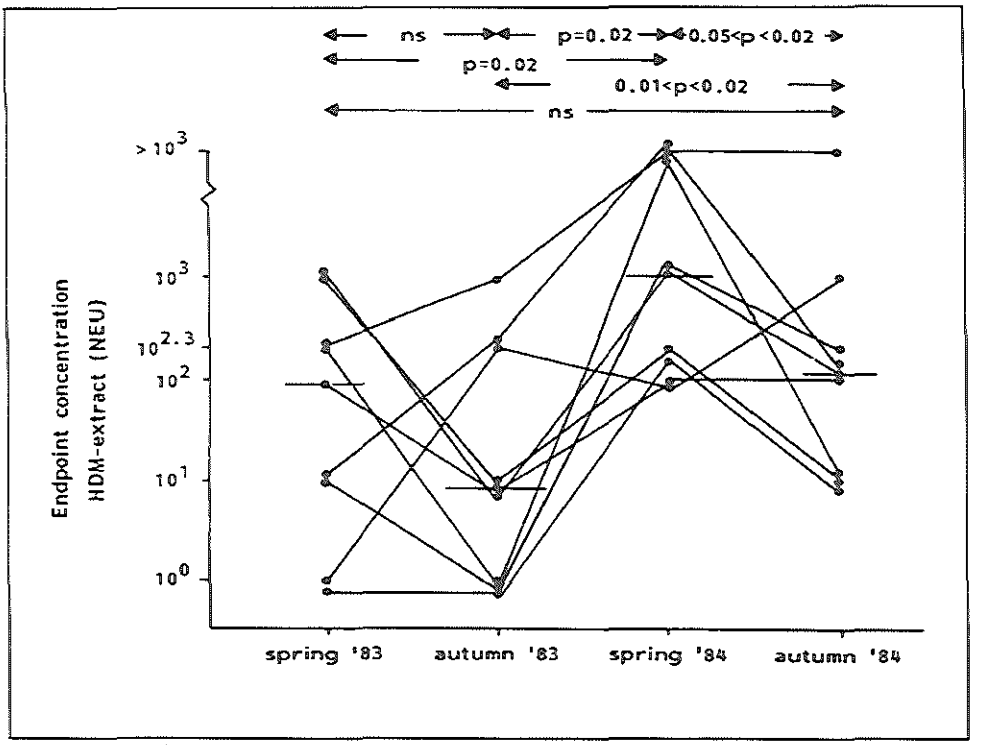

Figure 1. End-point concentrations in nasal provocation tests with HDM extract (NEU/ml) during the trial.

Table 2. Skin reactivity expressed in total number of standardized plus signs.

	spring	autumn	spring	autumn
skinreactivity	1983	1983	1984	1984
median	8	8	7	7
range	6-10	6-10	6-10	6-9
significance	---n.s.---	---n.s.---	---n.s.---	
	----------n.s.----------			
	----------n.s.----------			
	----------n.s.----------			

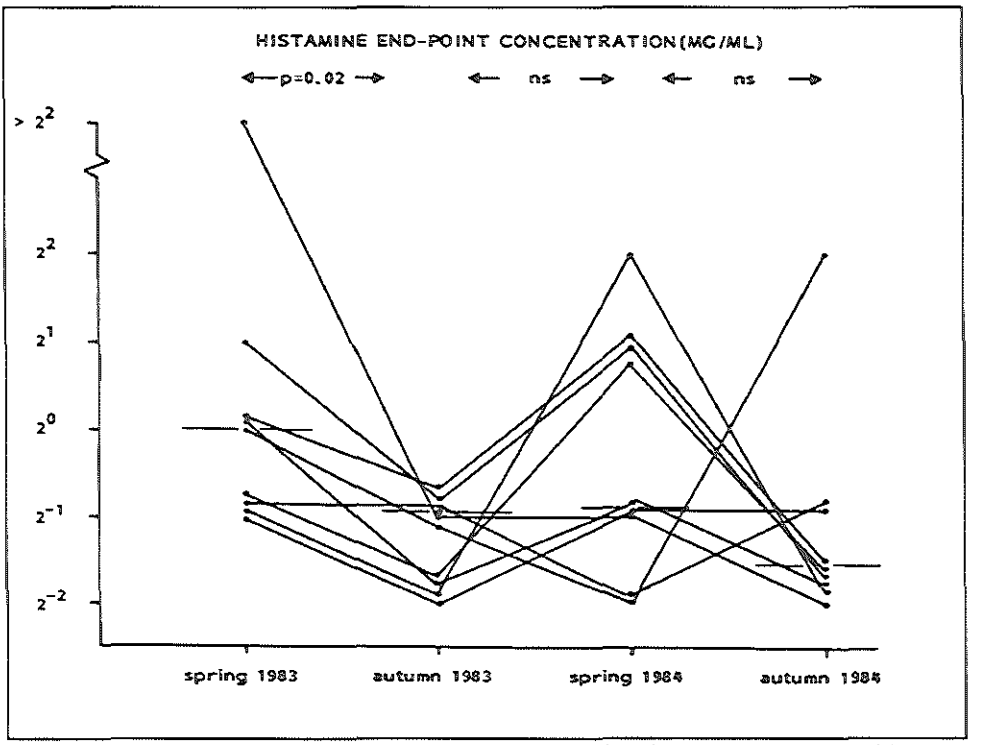

Figure 2. Endpoint-concentrations in nasal provocation tests with histamine (mg/ml) during the trial.

Table 3. RAST-results in arbitrary unitis (see text).

	spring	autumn	spring	autumn
RAST-results	1983	1983	1984	1984
median	8.9	12.0	18.2	16.0
range	3.0-36.6	4.2-22.8	3.5-43.6	3.4-41.7
significance	—n.s.—	---0.02<p<0.01—	—n.s.—	
	----------0.05<p<0.02----------			
		----------p=0.02----------		
	----------p=0.02----------			

DISCUSSION

In this study we monitored nasal responsiveness to HDM extract during immunotherapy. The measurement of the nasal resistance after provocation has been considered as the most objective way of assessing the nasal response (Mygind, 1982;Wihl, 1983). As there are three symptoms in nasal response (blockage, sneezing and secretion) we also took an

obvious amount of secretion or a certain number of sneezes as end-point.
We showed a marked fluctuation in nasal reactivity to HDM extract, with an increased sensitivity in autumn compared with spring of each year. Changes in nasal sensitivity to histamine were not so obvious except for the interval between Spring and Autumn 1983. In pollen allergy provocation tests are performed outside the season in order to avoid the influence of natural exposure to pollen on the nasal reacitivity to pollen extracts. The increase of sensitivity to histamine, methacholine and pollen during the grass pollen season is described by Borum et al.(1983).
Connell (1969) resported that daily provocation with ragweed pollen increases the sensitivity of the nasal mucosa (the "priming effect").
In this study the low end-point concentrations in the period August-October are probably due to the increased natural exposure to the house dust mite (Voorhorst et al., 1969), causing a priming effect.
Fluctuation in the outcome of provocation tests can be due to variation in baseline resistance (Mygind, 1983), however, this variation could be excluded in our patients. The fluctuation in nasal sensitivity could not be attributed to a fluctuation in allergy to HDM as no similar variation could be found in skin reactivity to HDM extract and in HDM-specific IgE. No conclusions can be drawn about the efficacy of immunotherapy with HDM extract as the study was not carried out in a double-blind, placebo-controlled fashion. However, the results of the trial imply that immunotherapy with HDM extract has to be evaluated with respect to the seasonal fluctuation in amounts of HDM.

CONCLUSION

In monitoring immunotherapy with HDM extract a fluctuation in sensitivity to HDM extract and possibly histamine was seen. Patients have an increase in nasal responsiveness to HDM extract in the period from August till October. This is probably due to the increased exposure to house dust mites in this period which has a priming effect. All evaluations of immunotherapy with HDM extract should thus take this seasonal fluctuation into account.

ZUSAMMENFASSUNG

Neun Patienten wurden 1,5 Jahre lang hyposensibilisiert mit einem Hausstaubmilbenextrakt. Halbjaehrlich wurde eine Nasenprovokation mit Histamin und Allergen ausgefuehrt. Die nasale Reaktion wurde an Hand der Veraenderung des Nasenwiderstandes, an Hand der Haeufigkeit des Niesens, und der Menge der Sekretion, die beide als Folge einer Provokation auftraten, gemessen.

Bei einem Vergleich der Resultate mit einem Abstand von einem Jahr (Fruehjahr 1983-1984, und Herbst 1983-1984), konnte eine Verminderung der nasalen Sensibilitaet gegenueber Allergen konstatiert werden. Die nasale Sensibilitaet gegenueber Hausstaubmilben war im Herbst groesser als im Fruehjahr desselben Jahres (1983 signifikant, 1984 nicht signifikant).

Schwankungen in Bezug auf die Histamin-Empfindlichkeit waren - ausser in der Periode vom Fruehjahr 1983 bis Herbst 1984 - weniger deutlich. Die konstatierten Fluktuationen konnten nicht einer "base-line" Variation des Nasenwiderstandes zugeschrieben werden. Man kann zur Konklusion kommen, dass eine saisonabhaengige Variation bezueglich der Empfindlichkeit gegenueber Hausstaub-milben die Resultate der Hyposensibilisierung beeinfluessen kann. Ausserdem muss man den Zeitpunkt der Auswertung der Therapie beruecksichtigen.

ACKOWLEDGEMENT

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CHAPTER 8. NASAL HYPERREACTIVITY AND LATE PHASE ALLERGIC REACTIONS IN GRASS POLLEN ALLERGY

(Submitted for publication)

Summary

To analyse the association between changes in nasal reactivity to histamine and the occurrence of a late nasal response, 10 patients with an allergy to grass pollen underwent a nasal challenge with histamine 24 hours before and 24 hours after a nasal provocation with grass pollen extract. Up to 10 hours after allergen provocation nasal lavage fluid was obtained to characterise early and late phase reactions by measuring the levels of histamine, LTC_4/D_4, as indicators of mediator release, and albumin, as a marker of increased vasopermeability. After allergen challenge with 100.000 SQU median levels of histamine in nasal lavage fluid increased from 6 to 11 ng/ml ($p=0.76$). LTC_4/D_4 and albumin levels significantly increased from 62 to 576 pg/ml ($p=0.008$) and from 15 to 81 mcg/ml ($p=0.008$) respectively.

Though one patient only showed a late phase reaction, 8 of the 10 patients showed an increase in reactivity to histamine after grass pollen challenge ($p=0.043$). We conclude that a decrease in nasal histamine threshold can occur after allergen provocation. However, a biochemically or clinically defined late phase response does not necessarily accompany this change in sensitivity.

INTRODUCTION

The association between hyperresponsiveness to non-specific stimuli and IgE-mediated allergic reactions in the lower airways has been established in previous studies (1,2). Cockcroft et al (2) showed that allergen provocation induced an increase in bronchial hyperresponsiveness to histamine and methacholine in allergic asthmatics. Moreover, they demonstrated that this increase in bronchial hyperresponsiveness is associated with the occurrence of a late phase allergic reaction (2).

Evidence that an association between allergen exposure and increase in nasal responsiveness to non-specific stimuli exists was already presented in 1960 by van Dishoeck and van Lier (3). They showed that during the grass pollen season nasal reactivity to veratrine (a mixture of alkaloids) increased in rhinitis patients allergic to this pollen. In the late sixties Connell (4) demonstrated an increase in nasal reactivity to ragweed and sorrel pollen after repeated exposure to ragweed pollen only. Connell called this local and temporary phenomenon 'the priming effect'. He attributed it to an increased permeability of the mucous membrane, which allows the allergen to penetrate more readily into the nasal mucosa. It has recently been established that the magnitude of this priming effect correlates strongly with the increase of nasal responsiveness to histamine (5).

Research into the association between nasal hyperreactivity and the existence of a late phase allergic reaction in the nose has been hampered by the methodological problems encountered in the assessment of the late nasal response. Although late-phase reactions have been observed by measurement of nasal airway resistance by Pelikan (6) and Dvoracek (7), Richardson was not able to demonstrate the existence of such a phenomenon (8). It is possible that the baseline variation in nasal airway resistance (9) and the presence of a nasal cycle (10) make determination of a late phase response in this way impracticable. Recently Mygind failed to demonstrate a clinical late phase response after allergen challenge (11).

Determination of the early and late phases has been made possible by the development of more sophisticated methods using the increase of mediators in nasal lavage fluid as markers of an allergic response after nasal challenge (12,13,14).

The aim of this study is to analyse the association between allergen-induced nasal reactivity to histamine and the presence of a late nasal response after allergen challenge.

The immediate and late nasal responses were measured in nasal lavage fluid by determination of histamine and leukotriene C_4/D_4 as indicators of mediator release from mast cells, basophils and other inflammatory cells. Albumin was determined as increased vasopermeability will lead to influx of albumin into lavage fluid.

METHODS

Study design

Rhinitis patients with an IgE-mediated allergy to grass pollen were challenged with both placebo and grass pollen extract on two different days (at 8.00 a.m.), out of the grass-pollen season (Fig 1). On both days nasal lavage fluid was obtained hourly for 10 hours after the provocation in accordance with the methods described by Naclerio (12). Nasal lavage fluid was analysed to determine histamine, LTC_4/D_4 and albumin levels. Twenty-four hours before and after the nasal challenge with grass-pollen patients underwent a challenge with histamine.

In order to evaluate the non-specific effects of the lavage procedure on nasal reactivity five other patients with allergic rhinitis underwent a placebo provocation with repeated lavages during the day. Twenty-four hours before and after placebo challenge a nasal provocation with histamine was performed.

The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam. All patients gave their informed consent before participating in the study.

Patients

Ten patients (six females and four males) took part in the study. Their ages ranged from 18 to 38 with a median age of 28 years. They were characterized by a typical history of seasonal rhinoconjunctivitis during the grass pollen season. All patients were skin-test positive when tested intracutaneously with a low concentration (10 SQU/ml) of grass pollen extract (ALK; obtained from Laboratorium Diephuis/ALK, Groningen). Five of the 10 patients also showed an allergy to house dust mites. The tests were performed out of the grass pollen season and at that time all of the patients including those allergic to house dust mites were free of symptoms or were stable without use of medicine. Patient characteristics are summarised in Table 1.

Table 1.

Patients	m/f	Age	Clinical characteristics	IgE mediated allergies*		
1	f	31	rhinitis	GP	HDM	
2	f	33	rhinitis	GP		
3	m	18	rhinitis	GP	HDM	
4	f	29	rhinitis	GP		
5	m	24	rhinitis	GP		
6	m	24	rhinitis	GP	HDM	
7	m	27	rhinitis	GP	HDM	BP
8	f	35	rhinitis,asthma	GP	HDM	
9	m	38	rhinitis	GP		
10	m	21	rhinitis	GP	HDM	
(allergic) Controls.						
1	m	30	rhinitis	GP		
2	f	22	rhinitis	HDM		
3	m	32	rhinitis	GP	HDM	
4	f	54	rhinitis	BP		
5	m	31	rhinitis	GP	HDM	CE

Patient characteristics. *: GP=grass pollen, HDM=house dust mite, BP=birch pollen, CE=cat epithelium

Five other patients, selected on the basis of their allergic rhinitis, took part in the control study (see Table 1). None were taking such medication as antihistamines, cromoglycate, topical corticosteroids or NSAIDs, which could influence the outcome of the challenges. None of the patients had undergone immunotherapy previously.

Nasal challenge with placebo or grass pollen extract and lavage technique

On each occasion the subjects waited half an hour prior to the test so that the nasal mucosa had time to acclimatise. On the placebo day a control solution (phosphate-buffered saline containing human serum albumin 0.03% and benzalkonium chloride 0.05%) was sprayed into the nostrils twice, with an interval of ten minutes, by means of a nasal pumpspray delivering a fixed dose of 0.125 ml solution, as described previously (15,16). On the allergen day grass polllen extracts of 10.000 and 100.000 SQU/ml (ALK) were applied.

Nasal lavage was performed in accordance with the protocol described by Naclerio (12,13). Nasal secretions were collected by lavage with 10 ml of saline, preheated to 37 C°. Five ml saline was instilled into each nostril. After 10 seconds lavage fluid was

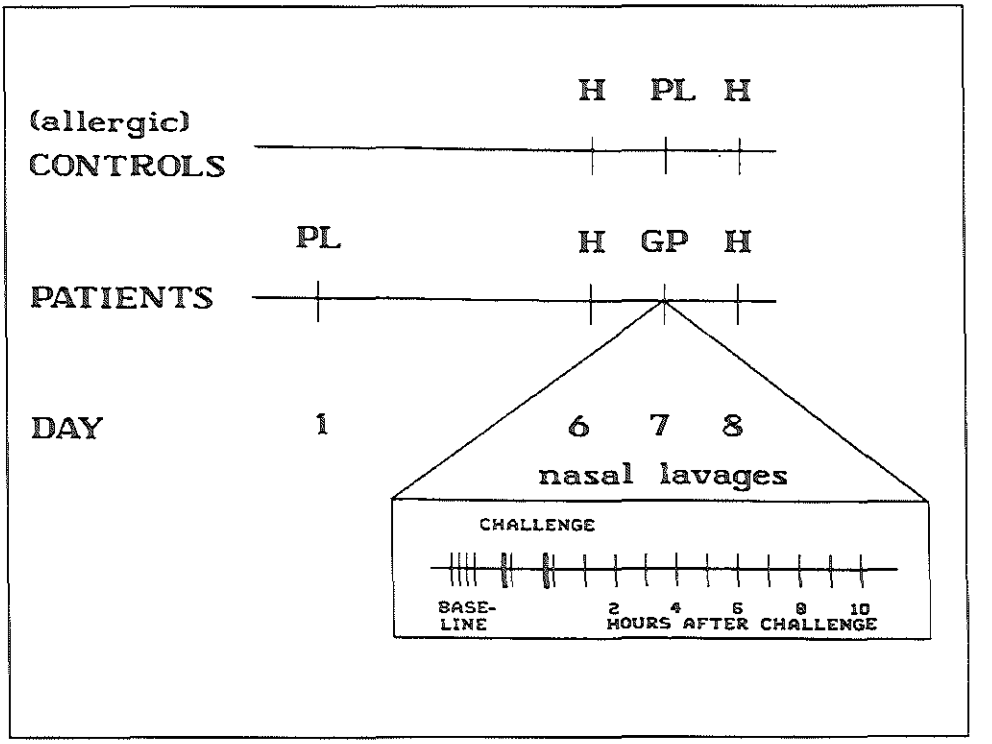

fig.1. Study design. Patients underwent a placebo (PL) provocation with repetitive lavages, a grass pollen challenge (GP) preceded and followed by a histamine challenge (H). Allergic controls underwent a placebo provocation (PL) which was preceded and followed by a histamine challenge. During the PL and GP days identical lavage protocols were followed.

expelled and collected in polystyrene tubes. The mean volume (+/- SD) of lavage fluid recovered was 7.8 +/- 1.0 ml.

The protocols were identical on the placebo and allergen days. They involved four prewashes to reduce mediator concentrations to baseline levels. The fourth prewash was used as the baseline fluid. Oxymetazoline 0.1 % (two 0.125 ml puffs) in each nostril was applied five minutes before the first provocation to prevent nasal congestion caused by the allergen challenges. Ten minutes after both provocations a nasal lavage was performed with saline. Nasal lavage fluid was collected hourly after the second provocation for ten hours.

Nasal challenge with histamine

Challenges were performed in accordance with the previously described methods (15,16). Histamine in concentrations of 0.25, 0.50, 1.0, 2.0 and 4.0 mg/ml was sprayed into each nostril at five-minute intervals after challenge with phosphate buffered saline. The amount of secretion was collected after each challenge using the method introduced by Borum (17). Secretion dripped into a funnel. The number of sneezes was counted.

Mediator assays

Lavage fluid was stored on ice and filtrated with a 5 mcrm cellulose nitrate filter (FP030/1;Schleicher & Schuell) within 60 minutes. Filtration was carried out to separate mucus and to obtain a clear fluid. Lavage fluid was stored at -20 °C until determination of the mediators.

In one portion of each sample the histamine was measured with an automated fluorometric assay (18) with a sensitivity of 1 ng/ml.

In addition, in samples obtained from two patients levels of methylhistamine were measured by Histamine RIA (Pharmacia). In this assay a monoclonal antibody is used that recognises both methylhistamine and histamine. With methylhistamine as a standard the cross-reactivity at 50% B/Bo for histamine (w/w) is 5.6% (information provided by Pharmacia).

As filtration of the fluid can damage cells with subsequent release of histamine, histamine in nasal lavage of three patients was also determined in the cell-free supernatant after centrifugation at 300 x g for 10 minutes (temperature: 4 °C) and filtration afterwards. From each patient 13 paired samples were obtained.

Four ml filtrated lavage fluid was applied to Amprep™ minicolumns (Amersham, UK) for the extraction of leukotrienes. Samples were eluted with 2.5 ml methanol 100%. Sulfidopeptide-leukotrienes were determined using RIA with antibody from Advanced Magnetics Inc and cross-reactivities at 50% B/Bo for LTC_4, D_4 an E_4 of 64%, 100% and 7% respectively.

^{3}H-LTC_4 (51 Ci/mmol) was obtained from Amersham, UK. Standard LTC_4 was donated by Dr. J. Rokach, Merck Frosst, Canada.

In addition, in samples obtained from two patients, levels of sulfidopeptide-containing-leukotrienes were measured using RIA from Amersham, UK, in which cross-reactivities

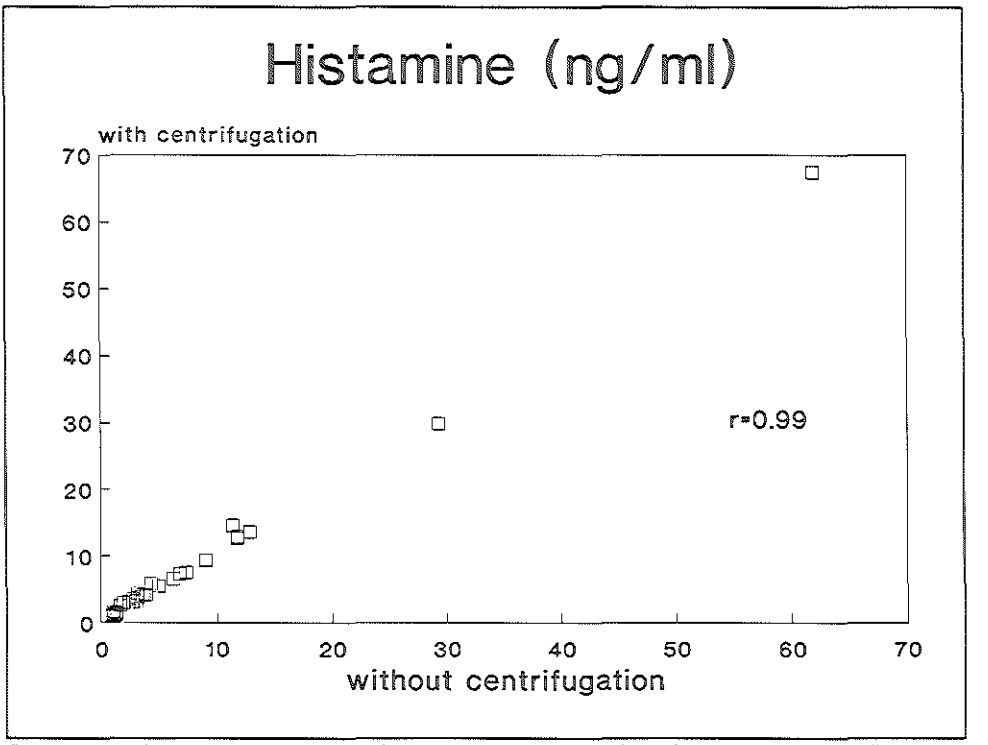

fig. 2. Histamine samples -with and without centrifugation- obtained from three patients. From each patient 13 paired samples were analysed.

were 100%, 46% and 64% for LTC_4, D_4 and E_4 respectively. Accordingly, antibody used in the latter assay has a nine times higher cross-reactivity to LTE_4 than the antibody used in the former.

Recovery after filtration and Amprep extraction was 84% +/- 3.5% for LTC_4.

Leukotrienes were determined using polypropylene disposables.

Albumin was estimated by radial immunodiffusion in agarose gel (RID) (19). The agarose gel contained 0.4 mcrl anti-albumin antiserum (Dako A001,Dakopatts,Copenhagen,Denmark) per cm^2.

Statistical analysis

In order to compare histamine levels measured with and without centrifugation a Spearman rank correlation was computed.

The levels of histamine, LTC_4/D_4 and albumin were not normally distributed. Therefore,

after placebo and allergen provocation these levels were compared with the baseline levels using the two-sided Wilcoxon signed rank test. In order to determine a biochemically characterised late phase reaction, peak levels of LTC_4/D_4, albumin and histamine which were found between 3 and 10 hours after provocation were compared with the baseline.

Histamine threshold values on the day before and the day after allergen provocation were compared by means of the Wilcoxon signed rank test. A p-value of 0.05 or less was considered statistically significant.

RESULTS

All patients underwent a nasal challenge with 10.000 SQU/ml grass pollen extract. One patient showed such a strong nasal response (>20 sneezes) after this challenge that provocation with 100.000 SQU/ml was not carried out. The other nine patients did undergo a nasal challenge with grass pollen 100.000 SQU/ml as well. These high concentrations were chosen as it has been suggested that late phase nasal reaction will only be induced by large doses of allergen (7). Both doses of allergenic extract induced a median number of sneezes of 3 (range:0-23) and 7 (range:0-10) respectively. The histamine, LTC_4/D_4 and albumin were determined in all 9 samples obtained after challenge with 100.000 SQU/ml.

Comparison of histamine levels with and without centrifugation obtained from three patients yielded a Spearman rank correlation of 0.99 (fig. 2). Therefore only measurements without centrifugation were taken in the 7 remaining patients.

The median levels of histamine, LTC_4/D_4 and albumin after challenge with 10.000, 100.000 SQU/ml and placebo are indicated in Table 2. On the allergen challenge day leukotriene and albumin levels had significantly increased 10 minutes after challenge with grass pollen extract compared with the baseline levels. The maximal histamine and albumin levels, measured between 3 and 10 hours after allergen challenge, had not increased in comparison with the baseline levels. In contrast a significant increase was seen in LTC_4/D_4 levels, but this increase was comparable with the small but significant increases in maximal mediator levels on the placebo challenge day. A comparison between the maximal levels of histamine, leukotrienes and albumin on both placebo and allergen challenge day yielded no significant differences.

Table 2.

Mediator	time	PL	GP
Histamine (ng/ml)	baseline	2 (1-4)	6 (3-17)
	challenge (placebo or 10^5 SQU/ml)	2.5 (2-6) p=0.78	11 (7-12) p=0.76
	maximum (3-10 u.)	4.5 (2-6) p=0.008	3 (2-4) p=0.21
LTC_4/D_4 (pg/ml)	baseline	90 (49-126)	62 (20-109)
	challenge (placebo or 10^5 SQU/ml)	88 (60-125) p=0.92	576 (233-1941) p=0.008
	maximum (3-10 u.)	144 (82-181) p=0.009	170 (96-250) p=0.007
Albumin (mcg/ml)	baseline	10 (5-17)	15 (11-22)
	challenge (placebo or 10^5 SQU/ml)	11 (8-20) p=0.68	81 (64-310) p=0.008
	maximum (3-10 u.)	21 (9-35) p=0.06	18 (8-46) p=0.1

Median levels of histamine, LTC_4/D_4 and albumin on the placebo day (PL) and allergen day (GP). In parentheses the 25-75 percentiles are shown. The p-value reflects the statistical significance of the difference between the levels after challenge and the baseline on the same day .

In only one patient (no.2;Table 1) was a biphasic response seen in histamine and albumin levels, whereas leukotriene levels did not return to the baseline after an early peak (fig 3 and 4).

The absence of a late-phase reaction in 9 of the 10 patients called for an experiment to determine the metabolites of histamine and sulfidopeptide-leukotrienes in the lavage fluid. Therefore in the patient with a biphasic response (fig. 3 and 4) and in another patient with a comparable strong immediate reaction, but without a late- phase reaction, methylhistamine and LTE_4 were determined. Low levels of methylhistamine were seen, particularly taking into account the cross-reactivity to histamine. Five percent of the corresponding histamine levels will be detected by the methylhistamine assay.

LTE_4 levels are depicted in fig.4. In fact LTC_4, LTD_4 and LTE_4 are determined with this assay, however the sensitivity to LTE_4 is 9-fold compared with the assay used in all patients. Although LTE_4 levels were higher than LTC_4/D_4 in both patients, the patterns

for both measurements were the same.

In order to evaluate the effect of allergen challenge on non-specific nasal reactivity, end-point concentrations of histamine required to induce 0.5 ml of secretion and/or at least 5 sneezes 24 hours before and after allergen challenge were compared. A significant decrease in end-point concentration was seen (fig.5). Whereas 8 out of the 10 patients showed a decrease in end-point concentration of histamine, only 1 of the 5 allergic control patients showed this decrease after placebo challenge with repeated lavages (fig.5).

DISCUSSION

In bronchial asthma an association exists between late-phase allergic reactions and an increase of bronchial hyperresponsiveness after allergen exposure (2). The main purpose of this study was to investigate whether allergen-induced nasal hyperreactivity is

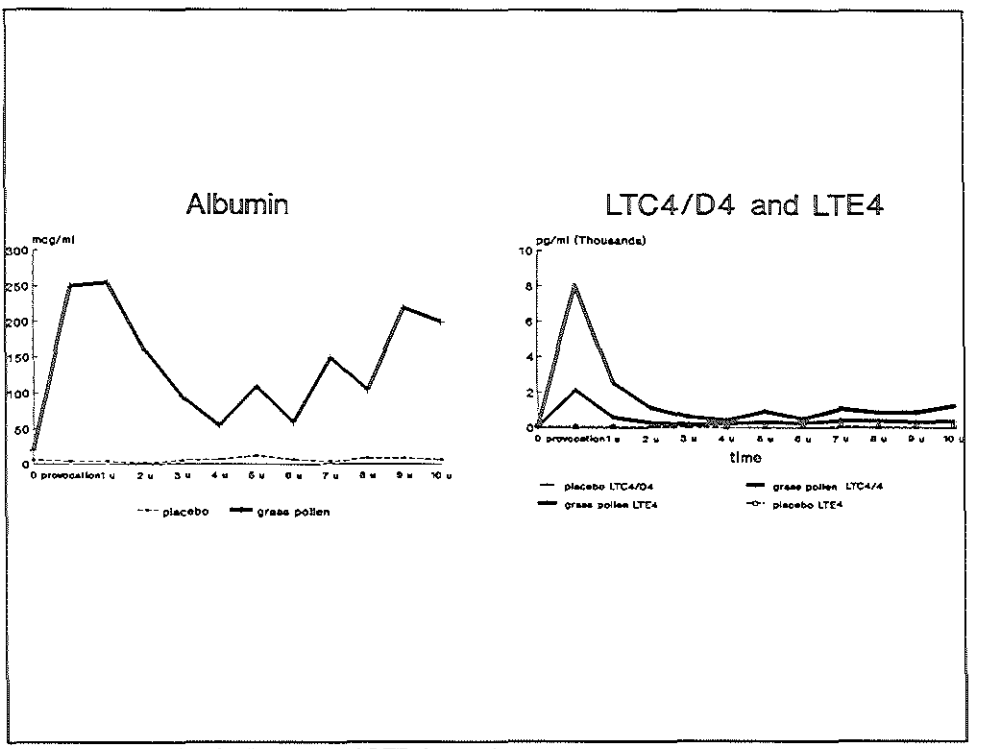

fig. 4. Levels of albumin, LTC_4/D_4 and LTE_4 in 1 patient.

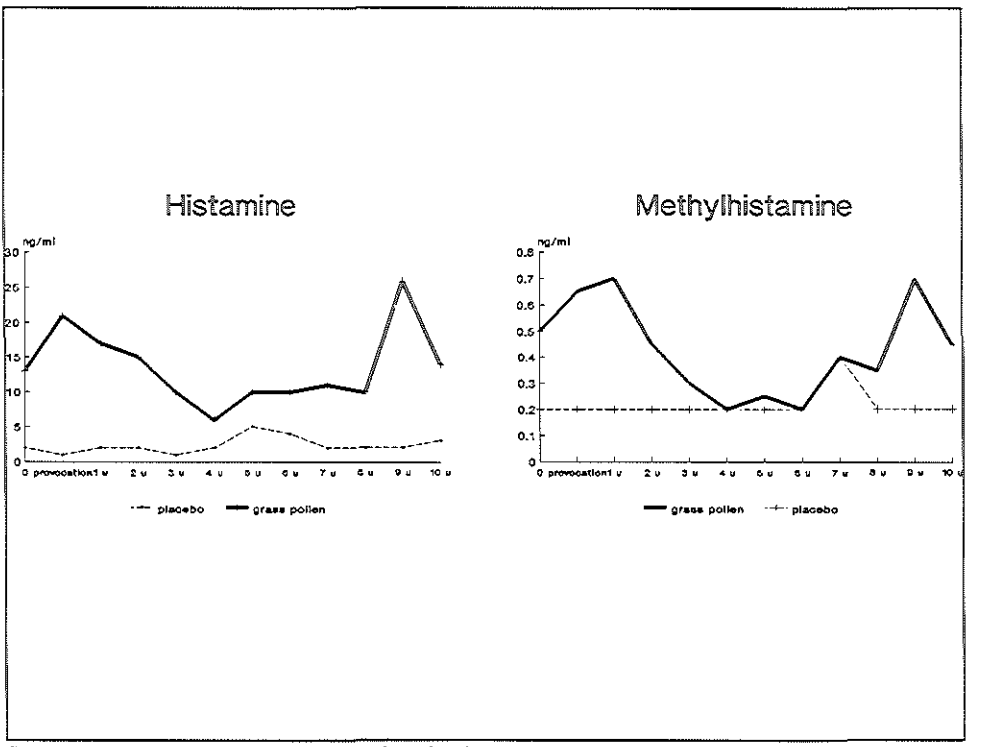

fig. 3. Levels of histamine and methylhistamine in 1 patient. Dotted lines represent the values on the placebo day. Straight lines depict the course on the allergen day.

accompanied by a late phase nasal reaction. Contrary to our expectations only one patient had a late-phase reaction. Moreover, levels of leukotrienes and albumin during the early reaction were lower than reported in other studies (12,14,22). In contrast histamine levels were of comparable magnitude with those reported elsewere (12). Several explanations of the absence of late reactions in the majority of our patients are possible. Patient selection was based only on existence of a grass-pollen allergy and not on occurrence of late-phase reactions after previous challenges as reported before (12). An important difference in our study is the use of allergenic extracts in the nasal provocation tests instead of whole pollen grains (12). It can be assumed, that in the latter case the release and resorption of allergen differ in character and time lapse from the quick exposure to and resorption of allergenic extract. The possibility that we overlooked late-phase reactions with our system is unlikely, as only the patient with a dual reaction experienced a recurrence of nasal symptoms (i.e. complaints of nasal

blockage) after a symptom-free interval. This is in line with the findings of Mygind who was not able to elicit a clinical late-phase reaction in patients provoked with grass pollen extract (11). Although characterisation of the immediate allergic reaction was not the main purpose of our study, the nasal symptoms after allergen challenge and the increases that were up to 41.4 and 10.3 times higher than the baseline levels of LTC_4/D_4 and albumin respectively, indicate that the amount of allergen used was sufficient. Therefore absence of late phase reaction could not be due to insufficient allergen exposure. In addition, determination of metabolites such as methylhistamine and LTE_4 gave no support to the assumption that we had overlooked late-phase reactions.
The cause of the significantly higher baseline level of histamine on the allergen-challenge day is not clear. It has been shown that bronchial provocation with histamine leads to a higher cell content (including mast cells) in bronchoalveolar lavage fluid 24 hours later (21). Therefore it is possible that a chemotactic effect of histamine used in the nasal provocation 24 hours earlier had induced higher levels of histamine from an increased cell debris. Our results suggest that histamine is a less appropriate marker than albumin and LTC_4/D_4 for the characterisation of the nasal allergic reaction in this model. This corresponds to the findings of Lebel (22), who showed that PGD_2 in lavage fluid correlates better with clinical symptoms than histamine does. In addition, Linder (23) demonstrated that histamine in lavage fluid decreases after allergen provocation, whereas Davies (24) reported that the increase of histamine after allergen challenge was not dose-dependent.
The small but significant increases in maximal levels of histamine, leukotrienes and albumin 3-10 hours after challenge on the placebo challenge day and the comparable increase in leukotrienes on the allergen challenge day suggest that baseline washings or diurnal variation might influence the results obtained with nasal lavage. These data indicate that 100 % increases in mediator levels must be interpreted carefully using this lavage protocol.
The patient with the late phase reaction did not show an increase of nasal responsiveness to histamine. In the other 9 patients without a late phase reaction, 8 showed an increase in nasal hyperreactivity. The assesment of nasal reactivity to histamine expressed in end-point concentrations to induce 0.5 ml of secretion and/or at least 5 sneezes proved to be reproducible (16) and therefore suitable in this study. Because of the time-consuming

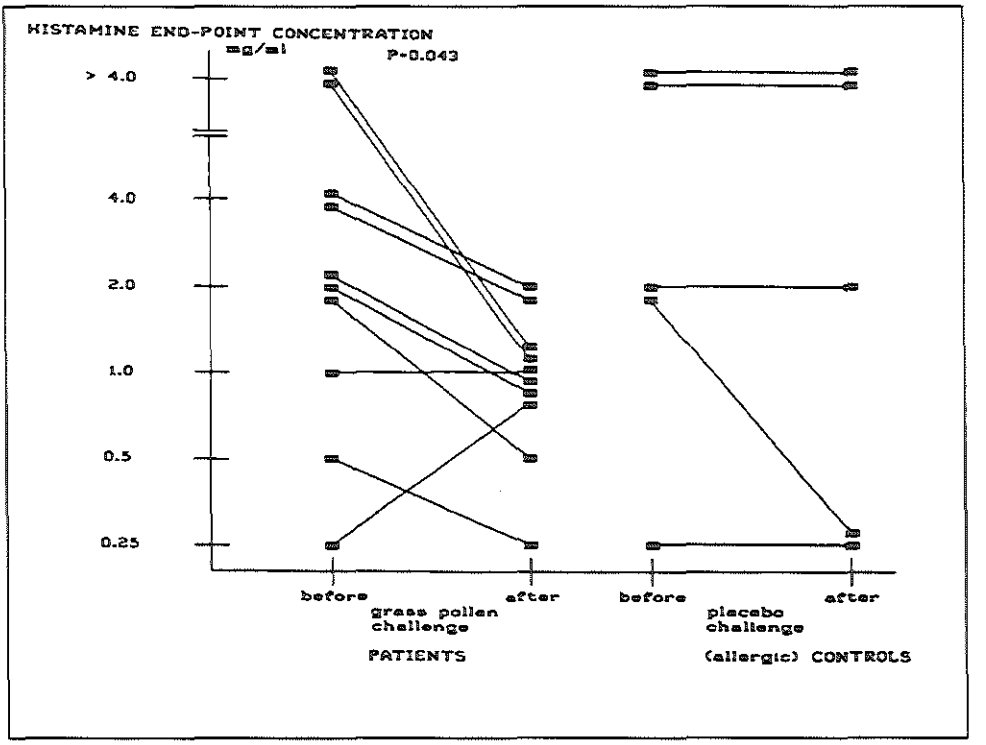

fig. 5. Histamine end-point concentration 24 hours before and after allergen challenge.

character of the protocol it was not possible to perform histamine challenges before and after the placebo provocation to evaluate the non-specific effects of the lavage protocol itself. However, in a second experiment in 5 other patients lavages as such did not increase nasal responsiveness to histamine in 4 subjects. This observation corresponds with other studies demonstrating that increase in nasal reactivity cannot be induced by non-immunological stimuli (25,26).

The findings of this study indicate that although allergen challenges lead to increase of non-specific nasal reactivity, a biochemically or clinically defined late phase reaction does not necessarily accompany this change in sensitivity.

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CHAPTER 9. NASAL ALLERGY TO AVIAN ANTIGENS

(As published in Clinical Allergy 1987;17:515-21)

SUMMARY

This study describes the case of a patient who developed symptoms of rhinoconjunctivitis on exposure to budgerigars and parrots. An IgE-mediated allergy to budgerigar, parrot and pigeon antigens was demonstrated using both in-vivo challenge tests (skin and nasal provocation tests) and in-vitro investigations (radio-allergo-sorbent test, histamine release test). The study shows that the development of nasal disease can be associated with allergy to avian antigens.

INTRODUCTION

The development of hypersensitivity pneumonitis in bird fanciers is well known. The disease was first described in pigeon breeders in 1965 by Reed et al (1). Exposure to other birds may also induce this disease (2,3,4,5). Type III and type IV allergic reactions may be involved (6). There is evidence that IgE-mediated mechanisms may also play a role in allergy to bird-derived materials (7,8,9). Isolated nasal allergy to avian antigens has rarely been reported. To our knowledge only Pelikan & Pelikan-Filipek (10) have analysed the nasal complaints in pigeon breeders.

SUBJECT AND METHODS

Mrs D, female, 47 years old, had a history of perennial rhinoconjunctivitis. Symptoms of running nose, sneezing and itching of the eyes had started 7 years previously and had gradually became worse. She had chronic symptoms with acute aggrevation immediately after contact with the budgerigar and the parrot, which she had been keeping for 8 years outside the house. This exacerbation continued for about 24 hours. She had no symptoms after contact with her pigeons. The history revealed no other provoking allergens. Other stimuli were non-specific (tobacco smoke, painty smell and fog). She had never experienced atopic dermatitis. No features of bronchial asthma or

Table 1. Intradermal skin tests

Allergen	concentration					
	0.00025	0.0025	0.025	0.25	mg/ml	(feather-extract)
	10^{-8}	10^{-7}	10^{-6}		10^{-5}	v/v (pigeon serum)
Budgerigar feather	+/-	+/-	+		+++	
Parrot feather	+	+	++		++	
Pigeon serum	-	+	++		++	

Skin test response of patient D, expressed according to the grading system of Norman (11). - Represents an erythema and weal of < 5 mm each; +/- reaction: erythema and wheal of 5-10 mm each; + reaction: erythema of 11-20 mm and weal of 5-10 mm each; ++ reaction: erythema of 21-30 mm and weal of 5-10 mm; +++ reaction: erythema of 31-40 mm and a weal of 10-15 mm or with pseudopods; ++++ reaction:erythema > 40 mm and weal > 15 mm or many pseudopods.

hypersensitivity pneumonitis were present. Spirometry, bronchial provocation with histamine and a chest X-ray gave normal results. There was marked blood eosinophilia ($0.673x10^9$ cells/l).

Allergens

Lyophilized budgerigar and parrot feather extracts and lyophilized pigeon serum were obtained from Diephuis Laboratorium, Groningen, The Netherlands.

Skin tests

Dilutions of budgerigar and feather extracts (0.25, 0.025 and 0.0025 mg/ml) and dilutions of pigeon serum (10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} v/v) were made using extract reconstituted in phosphate buffered saline (PBS) containing HSA 0.03% and phenol 0.5%. Intradermal skin tests were performed and reactions were read after 20 minutes using the grading system of standardized plus signs, devised

by Norman (11).

Nasal provocation tests

Lyophilized feather extract and serum were reconstituted with PBS containing HSA 0.03% and benzalkonium chloride 0.5%. After nasal provocation with 0.2 ml PBS in each nostril, dilutions of parrot or budgerigar feather extract (from 0.025 to 2.5 mg/ml in ten-fold steps), pigeon serum (from 10^{-5} to 10^{-2} v/v in ten-fold steps) or histamine phosphate (in dilutions of 0.25 and 0.5 mg/ml) were sprayed into each nostril by using a deVilbiss atomizer connected to a pressure pump. The allergens were applied at 15-minute intervals and histamine phosphate at 10-minute intervals. All tests were performed on separate days.

Total nasal airway resistance (NAR) was measured and calculated using a passive anterior rhinomanometer (Heyer Parr) as described by Clement et al (12). Sneezes were counted and secretion was collected as described by Borum (13).

Total IgE and allergen-specific IgE

Total IgE was determined using IgE RIA, (Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions. Allergen-specific IgE was determined by a radio-allergo-sorbent test using agarose beads as allergen support. Bird allergens were prepared and coupled to CNBr-activated Sepharose 4B as described previously (9).

Precipitating antibodies

Precipitating antibodies against feather extract from budgerigar and parrots, and against serum from pigeons, were determined by double diffusion using the method described by Ouchterlony (14).

Histamine release from washed leucocytes

Incubation of washed leucocytes with allergen extracts (budgerigar feathers, parrot feathers and pigeon serum) and assay of histamine released into the supernatant, were performed using Siraganian & Hook's method (15) as described previously (9).

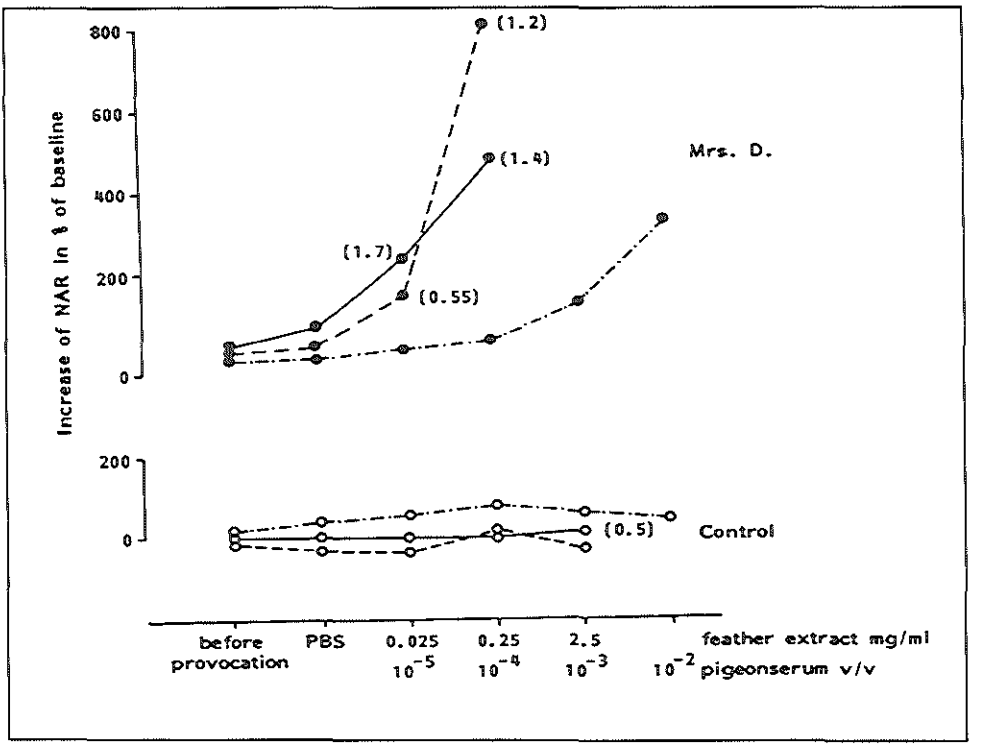

fig. 1. Increase in nasal resistance, expressed as percentage of the baseline value, induced by nasal provocation with allergen extract in Mrs D. (●) and a non-atopic control (○). Dose-response curves are constructed for budgerigar feather extract (●- - -●), parrot feather extract (●——●) and pigeon serum (● -•- ●). When a measurable amount of secretion was produced, the amount in ml is included in parentheses.

RESULTS

Skin tests

Intradermal skin tests with extracts of house dust mites, pollens, animal danders and moulds were negative. Mrs. D showed immediate type skin reactions to both budgerigar and parrot feather extract and to pigeon serum (table I). Non-specific effects of the extracts could be excluded as three non-atopic controls showed no skin reactivity to the avian antigens.

Nasal provocation tests

A marked increase of NAR was seen after provocation with avian antigens (Fig.1). This

nasal response could not be induced in a non-atopic control, indicating that the bird-derived materials that we used had no irritating effect on the nose. Histamine phosphate (0.25 mg/ml) caused an increase in NAR of 175% of the baseline value and produced 0.75 ml of secretion.

Total IgE and allergen-specific IgE

Serum obtained from the patient was tested for the occurrence of total and allergen-specific IgE. IgE against all feather extracts and the corresponding bird sera was found (Table II). In order to exclude non-specific binding to bird antigens, control studies were carried out by testing sera with total IgE levels between 300 IU/ml and 1300 IU/ml. A binding of 0-3 % was found (Table III). Control studies of the budgerigar feather RAST are described elsewere (9).

We looked for patient sera with IgE binding to parrot antigens, to find out whether this was an isolated phenomenon. In patient sera sent from other departments in the period 1984-1986 we found four sera containing IgE against parrot antigens (Table IV).

Precipitating antibodies

In Ouchterlony tests with serum from Mrs.D that was incubated with extracts of faeces or feathers or with serum from budgerigar, parrot or pigeon, there were either no precipitates or they were only weak.

Histamine release from washed leucocytes

The histamine release test showed that leucocytes obtained from the patient released histamine after incubation with budgerigar or parrot feather extract or pigeon serum, whereas a normal donor showed no release on exposure to these avian antigens (Fig.2).

Table 2.

IgE against avian antigens. Serum from Mrs D.

	Budgerigar		Parrot		Pigeon		total
	feathers	serum	feathers	serum	feathers	serum	IgE IU/ml
D.	17	15	25	21	5	15	500

Serum of Mrs. D. tested for the presence of IgE to budgerigar, parrot and pigeon feathers and serum. Results are expressed as binding of 125 I-anti-IgE.

Table 3.

Test for non-specific binding to bird antigens.

	Budgerigar		Parrot		Pigeon		total
	feathers	serum	feathers	serum	feathers	serum	IgE IU/ml
1	1	0					355
2	1	0					1350
3			0	0			365
4			3	1			5500
5					0	0	540
6					0	0	700

Six sera with various total IgE levels were incubated with Sepharose-coupled avian antigens.

Table 4.

IgE against parrot antigens. 4 patient sera.

Patient	Parrot feathers	serum	total IgE IU/ml
A.	9	0	1400
W.	15	1	450
Ro.	24	4	4000
Rij	27	32	340

Sera from 4 patients showing specific IgE to parrot feathers and sera.The results are expressed as binding of 125 I-anti-IgE.

DISCUSSION

Isolated nasal allergy to bird-derived antigens is uncommon. The literature on allergy to avian antigens has focused on the development of lung disease (1-7), especially on hypersensitivity pneumonitis (1-5). Pelikan & Pelikan-Filipek (10) recorded a late nasal response to pigeon dropping challenge in 53% of pigeon breeders with nasal complaints. Based on the occurence of precipitating IgG antibodies, general malaise, increased body temperature and a rise in white blood count, they suggested that a type III hypersensitivity reaction was involved.

The existence of IgE-mediated allergy has been established for budgerigars (7,9),canaries (9) and pigeons (8). Type I allergy to parrot antigens seems to be uncommon, as it is only recently that a patient with an allergy to parrots and also egg yolk has been described (16). The diagnosis of the allergy to the parrots was established by history and by RAST.

In this report we have demonstrated IgE against bird-derived material from several species. The findings of a recent study (9) suggest that budgerigar and canary feather extracts contain more different IgE binding antigens than the corresponding bird sera ,as only patient sera containing high levels of IgE against bird feather extract react with bird

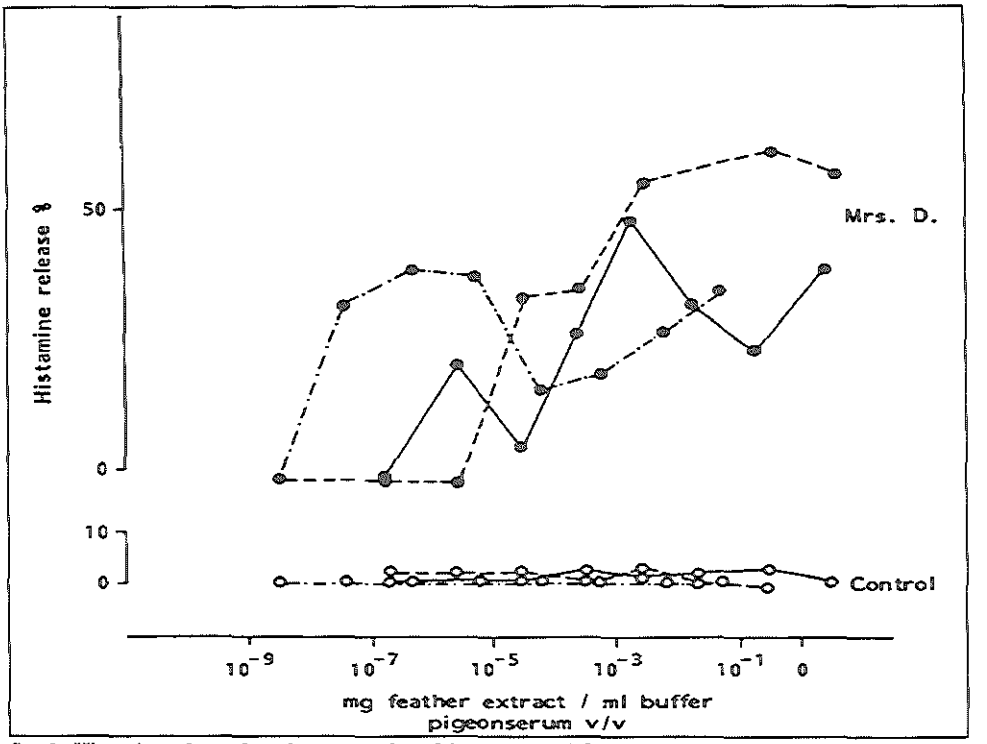

fig. 2. Histamine release from leucocytes from Mrs D. (●) and from a non-atopic control (○) on incubation with budgerigar feather extract (●- - -●),parrot feather extract (●——●) and pigeon serum (●-•-●).

serum. This is probably also true for parrot antigens , as all patient sera in this study (Tables II and IV) showed IgE binding in the parrot feather RAST that was higher than, or equal to, that corresponding serum RAST. However, the binding of IgE to pigeon feathers was less than the binding to pigeon serum. A possible explanation could be that a cross-reactivity exists between pigeon serum and other bird sera. It is also possible that we did not use an optimal allergen concentration in the pigeon feather RAST.

In the nasal provocation test, pigeon serum induced a significant nasal response (increase of NAR > 100%) in a concentration 10,000 times higher than the concentration needed for a 1+ skin reaction. The concentration of budgerigar feather extract needed to elicit a nasal response was 100 times the concentration needed for a 1+ skin reaction, while for parrot feather extract the concentration needed was equal to that for a 1+ skin reaction. This suggests that the sensitivity to pigeon antigen in the target organ ,the nose, was lower than the nasal sensitivity to budgerigar and parrot antigens, which in fact

corresponds with the patient's history.

In the nasal provocation test with histamine, Mrs D. reacted by producing a considerable amount of secretion when provoked with a low dose (0.25 mg/ml). We reported in a recent study that healthy non-atopic subjects do not show any nasal response after application of this concentration (17). This suggests the occurrence of a non-specific nasal reactivity. Evidence for a relation between allergy and nasal hyperreactivity has been produced by Borum (18), who reported an increase in reactivity to histamine and methacholine in pollinosis patients during the pollen season.

We conclude that rhinitis in bird fanciers can be associated with an IgE mediated allergy to bird antigens.

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PART IV. SUMMARY AND CONCLUSIONS

CHAPTER 10. SUMMARY AND CONCLUSIONS

Hyperreactivity or hyperresponsiveness in the nose as measured by nasal challenge with non-specific stimuli has been described since 1960 (van Lier 1960). Since then numerous nasal challenge studies have been performed. These studies have extensively been reviewed elsewere (Pipkorn 1989, Solomon & McLean 1989). Research into this pathological condition of the upper airways is hampered by a lack of standardised methods of measurement. In analogy to the methods used to determine bronchial hyperresponsiveness, nasal challenge tests with histamine and methacholine have often been used, despite the differences between bronchial and nasal tissue.

Measurement of nasal hyperreactivity

The first goal of the investigations reported in the previous chapters was to develop a method to measure nasal hyperresponsiveness (part II). A systematic approach was used, with

- evaluation of different application techniques,
- use of different agents (histamine, methacholine and phentolamine),
- assesment of different symptoms (sneezes, secretion and changes in NAR after histamine challenge, secretion induced by methacholine and increase in NAR induced by phentolamine),
- study of different patient groups (healthy subjects, allergic rhinitis, non-allergic perennial rhinitis and infectious rhinitis).

In chapter 3 we showed that the way of challenging can influence the outcome of the test. A nasal spray with saline when applied under pressure, can elicit a small but significant increase of nasal airway resistance (NAR) in allergic rhinitis patients compared with healthy subjects. Consequently, the nasal response to a test agent (non-specific or allergenic) may partly reflect a reaction to mechanical stimulation.
In the early investigations (chapters 7 and 9) we applied the test agents by spraying under pressure. It cannot be excluded that the results of nasal challenge with histamine and allergen are biased by this mechanical stimulation. However, this bias may be small as the pressure pump elicits a median increase in NAR of 17% above the baseline in

allergic rhinitis patients (chapter 2). Moreover, this method of application had an effect on NAR only, whereas in chapters 7 and 9 sneezes and secretion were also used in the determination of the nasal response. In the following studies (chapters 4,5,6 and 8) the more gentle nasal pumpspray without non-specific effects was used.

In chapter 4 nasal hyperresponsiveness to histamine, methacholine and phentolamine could be found in patients with allergic rhinitis compared with healthy subjects. The assessment of the reflex-mediated effects of histamine as measured by counting sneezes and determining the amount of secretion demonstrated a difference between the groups of allergic patients and healthy subjects. The assessment of vascular effects (increase in NAR) caused by histamine did not separate allergic patients and healthy subjects. The challenge with methacholine which has a stimulating effect on nasal glands, and phentolamine with only vascular effects also differentiated between the two groups, although these agents at least in the concentrations tested did not prove to be superior to histamine. In spite of differences between groups a considerable overlap in individual threshold values to the above mentioned agents could be observed. This overlap, found even in these selected patients, restricts the diagnostic value of these tests.

In another study (chapter 5) we divided non-allergic vasomotor rhinitis patients on anamnestic criteria into two subpopulations: the 'runners', characterised by a history of rhinorrhoea and sneezing and the 'blockers', characterised by a history of nasal blockage.
Both histamine by its reflex-mediated action and methacholine by its stimulating effect on glandular tissue were able to separate 'runners' on one hand from 'blockers' and healthy subjects on the other. Methacholine challenges discriminated better between 'runners' and healthy subjects than histamine did. With the test agents we used, it was not possible to discrimate between 'blockers' and healthy subjects.
In patients with infectious rhinitis hyperresponsiveness to non-specific stimuli has infrequently been the subject of investigation. Patients with chronic or recurrent bacterial infections were challenged with histamine, methacholine and phentolamine. Hyperresponsiveness to these agents could not be demonstrated. These findings correlated with the clinical observations: only 4 out of 19 patients had a history of nasal

complaints on non-specific stimuli.

The results of this study are in line with observations in bronchial asthma. Although viral infections induce increase of bronchial hyperresponsiveness and precipitate asthmatic attacks, bacterial infections of the lower airways do not provoke asthma (Busse 1990).

Pathophysiology

The studies described in chapters 4 and 5 were not designed to uncover the pathophysiological mechanisms of nasal hyperreactivity. However, a few remarks may be made. As the group of allergic rhinitis patients can be distinguished from healthy subjects by the reflex-induced action of histamine and the direct glandular stimulation by methacholine, both increased reflex and glandular activity may be responsible for nasal hyperreactivity in this patient group. Increased reflex and glandular activity could also explain the increased responsiveness to histamine and methacholine in the non-allergic 'runners' group.

Rhinitis patients and healthy subjects were challenged with phentolamine in order to reveal a defect in the α-adrenergic system. The use of phentolamine provocations has its origin in the study design employed with respect to the β-adrenergic system and asthma. In bronchial asthma hyperresponsiveness to propranolol (a β-adrenergic antagonist) has been interpreted as the result of a defect in the β-adrenergic system. A positive test reveals a tendency to a bronchial obstruction unopposed by circulating catecholamines when the β-receptors on the smooth muscle are blocked by propranolol (Koëter & Meurs 1984).

The in-vivo experiments in bronchial asthma were based on in-vitro receptor-binding studies. These studies - which investigated more specifically the α- and β adrenergic systems - revealed defects in the β-adrenergic system, in particular after allergen challenge (Koëter & Meurs 1984). In contrast, in the nasal mucosa receptor binding studies to α-adrenergic receptors yielded contradictory results. Van Megen (van Megen 1989) was unable to detect differences in density or affinity of α_1 recptors in the nasal mucosa obtained from allergic rhinitis patients and healthy subjects. Ishibe et al. (1983) and Konno et al. (1987), on the other hand, showed a decrease in α_1-receptor density in the nasal mucosa of the allergic patient.

Our study could be a confirmation of the latter investigation. However, the differences in responsiveness to phentolamine between allergic patients and healthy subjects - although significant - were small, which suggests a minor role of an α-adrenergic defect in this patient group.
Alternatively, the effects of the test agents could also be explained by an increased permeability of the nasal mucosa giving increased access for stimuli to sensory nerve endings, vessels and nasal glands. In particular, rapid changes in nasal hyperreactivity after exposure to allergen as demonstrated in chapter 8 could be based on changes in epithelial permeability. However, as histamine challenge has the same effect on the NAR in all patient groups and healthy subjects, increased epithelial permeability may not be a major mechanism. Similar evidence has been delivered by Stjärne et al. (1989). He demonstrated that capsaicin irritated the nose irrespectively of the subjects tested (vasomotor rhinitis patients and healthy subjects), whereas the (reflex-mediated) secretory response was increased in vasomotor rhinitis patients with predominantly symptoms of rhinorrhoea and sneezing. These differences in effect were attributed to hyperresponsiveness of muscarinic receptors and/or hypertrophy of the nasal glands, with an equal absorption of the test agents in healthy subjects and patients.
In conclusion, the data from chapters 4 and 5 suggest several explanations of nasal hyperreactivity. Apparently, reflexes and hyperresponsiveness of nasal glands are involved in the pathophysiology, whereas increased nasal permeability plays a minor role.

Clinical significance of nasal hyperreactivity

The second purpose of these investigations was to determine the clinical significance of nasal hyperreactivity and its role in allergic rhinitis (part III)

From previous studies on nasal hyperreactivity it is not known whether the nasal response after non-specific stimulation in the laboratory really reflects the 'clinical hyperresponsiveness' assessed by an accurate history.
In chapter 6 we were able to demonstrate that the reflex-mediated response of histamine challenge in allergic rhinitis is associated with anamnestic data obtained from the patient. A close association was observed between the outcome of the histamine challenge and a

hyperreactivity score estimating the sensitivity to everyday stimuli. In addition, the histamine challenge was associated with the current intensity of symptoms scored during the six days preceding the test.
So, nasal hyperreactivity to histamine is associated with 'clinical hyperreactivity', assessed by a careful history.

Links between hyperreactivity and allergen exposure

In bronchial asthma the link between hyperreactivity and allergen exposure has been established. In particular the increase of hyperreactivity after allergen exposure has been associated with the occurrence of a late phase allergic reaction (Cartier et al. 1982). In two studies we also demonstrated that allergen exposure (chapter 7) and allergen provocation (chapter 8) increased nasal hyperreactivity.

In previous studies (Borum et al. 1983) an induction of nasal hyperreactivity during natural pollen exposure was demonstrated. The influence of natural house dust mite exposure on nasal sensitivity to allergen and non-specific stimuli has never been investigated. In patients with a house dust mite allergy (chapter 7) we could show an increase in nasal sensitivity to house dust mites in the period from August till October in both years of the study. In this period natural exposure to house dust mites is highest (Voorhorst et al. 1969). Statistically significant increase in nasal hyperreactivity could be observed only in the autumn of the first year of the study. However, the majority of patients showed an increased hyperreactivity in the autumn of both years compared with the corresponding spring: nine and eight, respectively, out of nine showed a low threshold concentration of histamine (0.25 or 0.5 mg/ml) in the autumn of the first and second year, whereas in spring four and five respectively, demonstrated such a low end-point concentration. These observations suggest an influence of allergen exposure on nasal hyperreactivity. Conceivably, this seasonal fluctuation might reach statistical significance if the number of patients were larger. Also, the measurement of NAR besides sneezes and secretion in the assessment of histamine challenge test might have diminished fluctuations as the latter two variables are more closely associated with the symptomatology of the patient than the NAR (chapter 6).

In bronchial asthma, the importance of the late phase reaction has been emphasized. This late phase and the concomitant cellular involvement have been linked with the induction of bronchial hyperresponsiveness.
In the nose a late phase allergic reaction and a time-related cellular influx have been demonstrated, but their roles in the pathogenesis of allergen-induced nasal hyperreactivity have not been established yet.
In chapter 8 we describe the influence of nasal challenge with grass pollen extract on non-specific nasal reactivity to histamine in a group of 10 patients allergic to grass pollen. As expected we were able to induce an increase in nasal hyperreactivity by allergen challenge. This effect was not associated with a late phase allergic reaction characterized by mediator release and recurrence of nasal symptoms. Thus, in the nose an isolated immediate reaction is sufficient to induce nasal hyperreactivity.
The two studies described in chapters 7 and 8 demonstrated the connection between nasal allergy and hyperreactivity. In chapter 9 a case history illustrates that nasal allergy and hyperreactivity may coincide. This study was designed to describe the occurrence of IgE-mediated nasal allergy to avian antigens derived from birds such as budgerigars, parrots and pigeons in a bird fancier. Nasal hyperresponsiveness to histamine together with a clinical history of complaints on exposure to non-specific stimuli were also present.

The studies described in chapters 7,8 and 9 cannot answer the question concerning which cell or mediator is responsible for allergen-induced hyperreactivity. A first step to connect inflammation and nasal hyperreactivity implied research to late phase reactions in relation to allergen-induced nasal hyperreactivity. We could find no such relationship, but we have not examined the patterns of cell influx either in subjects with an isolated single response or in dual responders.
Therefore further studies of the pathophysiology of allergen-induced nasal hyperreactivity may involve determination of cells in connection with nasal hyperreactivity or nasal application of mediators such as leukotrienes, PAF and use of their antagonists.

Paralells with bronchial hyperresponsiveness

As discussed in the introduction, a considerable overlap in non-specific hyperresponsiveness between asthmatic patients and healthy subjects has been observed (Casale et al. 1988, Popa et al. 1988). In a large community-base sample of 2000 children bronchial challenge testing could not reliably separate asthmatics from nonasthmatics (Pattemore et al. 1990). In contrast, selected asthmatic patients can clearly be distinguished from healthy subjects (Cockroft et al. 1977). The PC_{20} of histamine in asthmatic patients is 30 to 40 times less than the PC_{20} in healthy subjects.

In the studies described in this thesis an overlap in nasal hyperreactivity also demonstrated when comparing rhinitis patients and healthy subjects. The discrimination between allergic rhinitis patients and healthy subjects was better than that reported in other studies (McLean et al. 1977, Guercio et al. 1979, Okuda et al. 1985). However, the eightfold difference in histamine endpoint between patients and normal persons is less pronounced than the differences seen in asthmatics and nonasthmatics.

These observations might be explained by differences in the pathophysiology of nasal and bronchial hyperresponsiveness.

As in nasal hyperreactivity various pathophysiological explanations have been put forward as responsible mechanisms for bronchial hyperresponsiveness (Sterk & Bel 1989). Some of the mechanisms that are potentially involved in bronchial hyperresponsiveness might be important in nasal hyperreactivity also. Examples are: epithelial damage or malfunction, neural control, inflammatory cell number and function. Other explanations such as increased smooth muscle contractility or reduction in viscous or elastic loads on airway smooth muscle cannot be transferred to the nose. It is possible that the absence of smooth muscle in the nose is responsible for the less pronounced difference in nasal hyperreactivity between patients and normal persons.

By bronchial challenge tests with different nonspecific stimuli distinct hyperreactivity patterns can be demonstrated in asthmatic and bronchitic patients (Gökemeijer 1976). It has been assumed that these differences in response patterns to non-specific stimuli might be a reflection of a distinct pathophysiology in these patient groups. This concept may be applicable to the groups of rhinitis patients (allergic patients, non-allergic runners and blockers, and infectious rhinitis patients) which displayed different response patterns to histamine, methacholine and phentolamine.

Final conclusions

The results and conclusions of the series of investigations described in chapters 2-8 can be summarised in some final conclusions:

1 Measurement and pathophysiology of nasal hyperreactivity

We know less about nasal hyperreactivity than about nasal immunology. The basic mechanisms of nasal hyperreactivity are poorly understood. In this respect more research has to be done into the direct relation between epithelial permeability, cell receptor populations, inflammation and hyperreactivity.

The measurement of nasal airway resistance (NAR) in histamine challenges does not differentiate between patients and healthy subjects. Moreover, NAR is not associated with the symptomatology of patients. Consequently, rhinomanometry is not suitable for assessment of histamine challenges.

With respect to the reproducibility of a nasal histamine challenge the use of reflex-mediated symptoms such as sneezing and secretion is advocated in the assessment of the nasal response. The use of NAR measurements yields a lower reproducibility.

The results of a nasal challenge test may be biased by the method of application: Mechanical stimulation of the nasal mucosa in a standardised way could serve as a method of assessing nasal hyperreactivity.

The pathophysiology of nasal hyperreactivity to non-specific stimuli is not clear. Increased reflex and glandular activity might be responsible for hyperreactivity, whereas increased epithelial permeability appears to be of minor importance. In allergic rhinitis α-adrenergic dysfunction may occur as well.

The term 'nonspecific' hyperreactivity is not correct, as some patients ('runners') react to nasal stimulation with histamine and methacholine, but not

with phentolamine.

2 Diagnostic value of nasal provocation tests with non-specific stimuli

Although nasal challenges with non-specific stimuli may distinguish patient groups and healthy subjects, the diagnostic value of these test to determine nasal hyperresponsiveness is limited in the individual patient, because of the overlap in threshold concentration between the patients and healthy subjects.
Nasal challenge tests with non-specific stimuli in patients with non-allergic vasomotor rhinitis can only distinguish a subgroup of patients with a clear-cut history of sneezing and running nose. Therefore, these tests have no function in the diagnosis or treatment of non-allergic vasomotor rhinitis.

3 Non-allergic vasomotor rhinitis

The occurrence of distinct response patterns to non-specific stimuli in patients with non-allergic vasomotor rhinitis points at a heterogeneity in this group of patients. This heterogeneity might be responsible for the contradictory findings in studies of nasal hyperreactivity in non-allergic rhinitis.

4 Infectious rhinitis

In bacterial infectious rhinitis hyperreactivity of the nasal mucosa does plays no part.

5 Allergic rhinitis

In allergic rhinitis the nasal response to histamine is closely associated with the symptomatology. Therefore this test might be a useful tool in the monitoring of allergic rhinitis patients with respect to therapeutical intervention such as allergen avoidance, medical treatment and immunotherapy.

The influence of allergen exposure may have detrimental effects on longterm variability in nasal hyperreactivity. However, the rapid changes in nasal sensitivity to non-specific stimuli under influence of allergen challenge indicate that nasal hyperreactivity might serve as a marker of allergen exposure.

Increased nasal hyperresponsiveness might be a predictor of the response to allergens. It is therefore possible that histamine challenge tests in combination with skin tests may be a substitute of allergen challenge tests. Vice versa the outcome of an allergen challenge test will be biased by nasal hyperreactivity. The influence of nasal hyperreactivity on the nasal response to allergen is currently under study.

The fluctuation in allergen exposure has to be taken into account when challenging rhinitis patients with a house dust mite allergy.

The clinical significance of the late phase reaction to allergens in the nose remains to be established, as the late phase reaction is not required to induce hyperreactivity.

The induction of nasal hyperreactivity in the absence of a late phase reaction might imply that the cellular influx observed during the late nasal reaction is not a prerequisite for the induction of nasal hyperreactivity.

In bronchial asthma an interaction between allergic reaction and bronchial hyperresponsiveness has been described. In allergic rhinitis the allergic reaction and nasal hyperreactivity may be related as presented in figure 1.

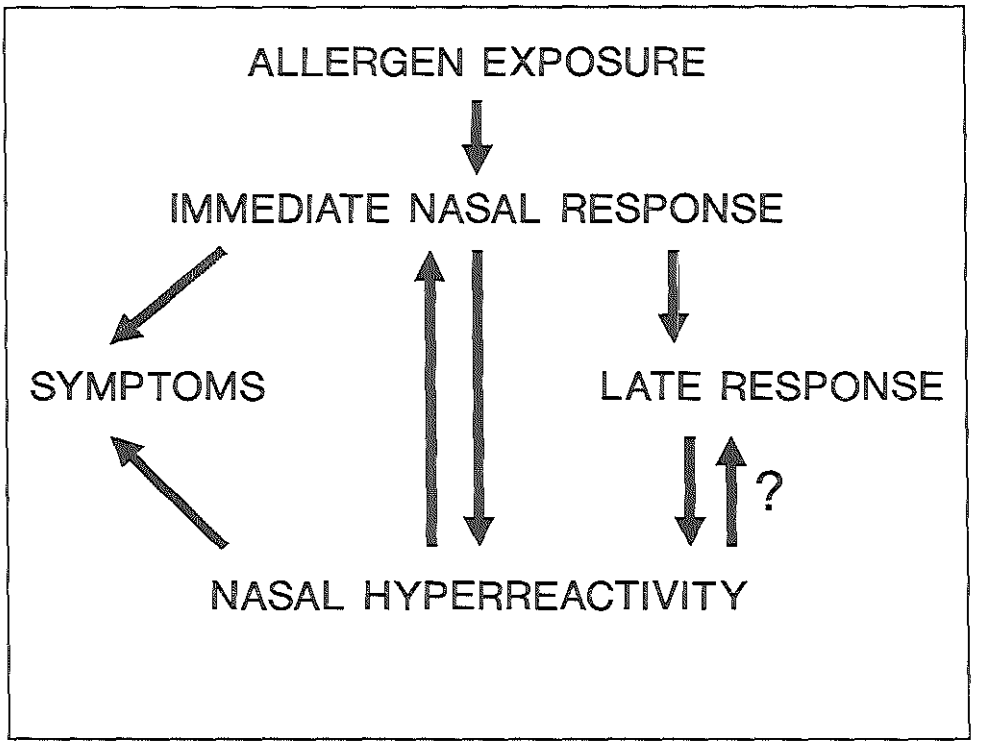

fig. 1

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CHAPTER 11. SAMENVATTING EN CONCLUSIES

Inleiding

Hyperreactiviteit van de nasale mucosa wordt gedefinieerd als een toegenomen gevoeligheid van het neusslijmvlies voor aspecifieke stimuli. Een dergelijke gevoeligheid voor stimuli, zoals temperatuursovergang, de geur van parfums en tabaksrook, uit zich in symptomen van niezen, loopneus en neusverstopping. Deze aspecifieke prikkels geven in het algemeen bij gezonde personen geen aanleiding tot neusklachten.

Naar analogie van technieken, waarmee bronchiale hyperreactiviteit wordt vastgelegd, is het gebruikelijk om - ondanks de verschillen tussen bovenste en onderste luchtwegen - de nasale hyperreactiviteit te meten door middel van applicatie in de neus van farmaca, als histamine of methacholine.

Twee vraagstellingen vormen het uitgangspunt van dit proefschrift:

1. Wat is de beste meetmethode van een nasale hyperreactiviteit?
2. Wat is de klinische betekenis van nasale hyperreactiviteit in relatie tot allergische rhinitis?

In hoofdstuk 2 wordt een overzicht gegeven van de literatuur over nasale hyperreactiviteit, IgE-gemedieerde allergie en de relatie hiertussen. Uit dit overzicht wordt een aantal conclusies getrokken.

Allereerst bestaat er een grote variatie in meetmethoden om hyperreactiviteit in de laboratoriumsituatie vast te leggen.
Het is onduidelijk welke meetmethode de beste is en in hoeverre bevindingen bij de ene onderzoekspopulatie (b.v. patiënten met een allergische rhinitis) kunnen worden geëxtrapoleerd naar andere groepen (patiënten met niet-allergische rhinitis vasomotoria en patiënten met een infectieuze rhinitis). Voorts bestaat er nog weinig inzicht in de pathofysiologie van nasale hyperreactiviteit. Ook is nooit nagegaan in hoeverre "klinische" hyperreactiviteit, vastgelegd aan de hand van een zorgvuldige anamnese of

van klachtenscores, en "gemeten" hyperreactiviteit, bepaald met behulp van nasale provocatietesten, met elkaar samenhangen.

Tenslotte is uit de literatuur wel duidelijk, dat IgE-gemedieerde allergie en nasale hyperreactiviteit met elkaar kunnen samenhangen. Echter is onbekend hoe deze allergeen-geinduceerde hyperreactiviteit tot stand komt. Onderzoekingen naar de rol hierbij van bepaalde celpopulaties en mediatoren zijn meestal alleen beschrijvend van aard en kunnen de pathofysiologie niet verklaren. Met name is onduidelijk, in hoeverre de late allergische reactie, die in de lagere luchtwegen gecorreleerd is aan toename van de bronchiale hyperreactiviteit, samenhangt met hyperreactiviteit van de nasale mucosa.

Meting van nasale hyperreactiviteit

In dit proefschrift werden meetmethoden van nasale hyperreactiviteit systematisch onderzocht aan de hand van:

- evaluatie van verschillende applicatietechnieken
- gebruik van verschillende stimuli als histamine, methacholine en phentolamine,
- het vastleggen van verschillende symptomen:
  - niezen, secretie en toename van neuswegweerstand (NAR) na histamineprovocatie,
  - secretie na methacholine-provocatie,
  - toename in NAR na phentolamine-provocatie,
- onderzoek bij verschillende groepen:
  - patiënten met allergische rhinitis,
  - niet-allergische rhinitis vasomotoria,
  - infectieuze rhinitis en
  - gezonde personen.

In hoofdstuk 3 werd aangetoond, dat de wijze van provocatie op zich van invloed kan zijn op de testresultaten. Mechanische verneveling van een vloeistof onder druk geeft aanleiding tot een lichte toename in NAR (17% van de uitgangswaarde) bij patiënten met allergische rhinitis. Derhalve kan de nasale respons op een testvloeistof voor een klein deel het gevolg zijn van rechtstreekse stimulatie van het neusslijmvlies. In de eerste

onderzoekingen van dit proefschrift (hoofdstuk 7 en 9) werd gebruik gemaakt van deze wijze van provocatie. Echter zal deze applicatiemethode de conclusies van deze studies nauwelijks beinvloeden, gezien de geringe invloed op de NAR in absolute zin. Bovendien worden in deze onderzoekingen ook andere symptomen, als niezen en neussecretie, gebruikt om de hyperreactiviteit mede vast te leggen. In de andere onderzoekingen (hoofdstuk 4, 5, 6 en 8) werd gebruik gemaakt van een eenvoudige neusspray zonder effect op de NAR.

In hoofdstuk 4 wordt de nasale reactiviteit voor histamine, methacholine en phentolamine bij patiënten met een allergische rhinitis vergeleken met die bij gezonde personen. De patiënten onderscheidden zich als groep van de gezonde personen door een toegenomen reactiviteit op histamine, gemeten aan de hand van reflex-gemedieerde symptomen, als niezen en secretie. Door bepaling van de neuswegweerstand was het niet mogelijk om de patiënten van gezonde personen te onderscheiden. Het bleek ook mogelijk om door provocatie met methacholine (secretie) en met phentolamine (neuswegweerstand) de groep patiënten en de groep gezonde personen van elkaar te onderscheiden. Ondanks de significante verschillen tussen de 2 groepen werd tussen beide groepen een overlapping in individuele drempelbepalingen voor deze drie stoffen gevonden.

In hoofdstuk 5 wordt een onderzoek beschreven bij patiënten met niet-allergische rhinitis vasomotoria. Op grond van de anamnese konden deze patiënten in 2 groepen worden onderverdeeld : in "runners" met een anamnese van loopneus en/of niezen, en "blockers", voornamelijk gekarakteriseerd door stoornissen in de neuspassage. De "runners" onderscheidden zich zowel van de gezonde personen als van de "blockers" door een toegenomen reflex-gemedieerde activiteit en een secretoire respons na methacholine-provocatie.

Bij patiënten met infectieuze rhinitis, dus met chronische of recidiverende bacteriële infecties in de neus, is zelden onderzoek naar nasale hyperreactiviteit verricht. In hoofdstuk 5 worden de resultaten van neusprovocatie met histamine, methacholine en phentolamine bij een dergelijke groep patiënten beschreven: hyperreactiviteit kon niet worden aangetoond.

Uit de studies van hoofdstuk 4 en 5 kan worden geconcludeerd, dat nasale hyperreactiviteit bij allergische rhinitis gepaard gaat met verhoogde reflex-activiteit van de nasale mucosa. Ditzelfde is het geval bij de "runners".
Bij allergische rhinitis speelt mogelijk ook een dysfunctie van het α-adrenerge zenuwsysteem in de neus een rol. De gevonden hyperreactiviteit voor phentolamine - een α-adrenerge antagonist - bij patiënten met allergische neusklachten wordt als een mogelijk gevolg hiervan beschouwd. Aangezien applicatie van histamine bij rhinitispatiënten en gezonde personen eenzelfde toename van neuswegweerstand induceert, lijkt de eventueel toegenomen permeabiliteit van epitheel bij rhinitispatiënten geen belangrijke rol te spelen.

Klinische betekenis van nasale hyperreactiviteit:
De tweede vraagstelling van dit proefschrift betreft de klinische betekenis van nasale hyperreactiviteit bij allergische rhinitis. Uit eerdere studies kan niet worden opgemaakt in hoeverre gemeten nasale hyperreactiviteit voor histamine en dergelijke samengaat met "klinische" hyperreactiviteit, geschat aan de hand van een nauwkeurige anamnese of een bijgehouden klachtenscore.
In hoofdstuk 6 wordt weergegegeven hoe wel een verband kon worden gelegd tussen de reflex-gemedieerde reactie op histamine-applicatie en anamnestische gegevens van de patiënten. Het bleek, dat een anamnestische "hyperreactiviteitsscore" een voorspellende betekenis had voor de mate van reactie op in de neus geappliceerde histamine. De reactie op histamine bleek tevens sterker bij toegenomen intensiteit van de neusklachten ten tijde van de neusprovocatietest. In hetzelfde onderzoek werd aangetoond, dat de reproduceerbaarheid van reflex-gemedieerde symptomen door histamine beter was dan die van de veranderingen in neuswegweerstand.
Bij astma is bekend, dat een verband bestaat tussen allergie en bronchiale hyperreactiviteit in die zien, dat allergeenexpositie leidt tot toename van de hyperreactiviteit. In twee studies (respectievelijk hoofdstuk 7 en 8) kon worden aangetoond, dat natuurlijke allergeenexpositie en neusprovocatie met allergeenextracten aanleiding geven tot toename van nasale hyperreactiviteit.
Bij rhinitispatiënten met een allergie voor huisstofmijten bleek de gevoeligheid van het neusslijmvlies voor huisstofmijtenextract toegenomen te zijn in de periode van augustus

tot oktober gedurende beide jaren van de studie. In deze periode is de natuurlijke expositie aan huisstofmijten-allergeen het grootst. Alleen in het eerste jaar van de studie werd een significante toename van de gevoeligheid voor histamine in deze periode waargenomen. In het tweede jaar van de studie was alleen een niet-significante fluctuatie waarneembaar. Het is mogelijk, dat deze fluctuatie statistisch significant zou zijn bij grotere aantallen patiënten. Bij dit onderzoek werden alleen veranderingen in neuswegweerstand vastgelegd. Indien bij de beoordeling ook het aantal niezen en de mate van secretie betrokken hadden kunnen worden, waren wellicht de fluctuaties in histaminegevoeligheid tussen najaar en voorjaar duidelijker geworden.

In hoofdstuk 8 wordt de invloed van provocatie met graspollenextract op de nasale hyperreactiviteit - gemeten aan de hand van provocatie met histamine - beschreven in een groep van 10 patiënten met een pollinose. Zoals verwacht, werd een toename van de hyperreactiviteit voor histamine onder invloed van de provocatie met allergeenextract waargenomen. Het bleek, dat een geisoleerde directe reactie op allergeenextract voldoende was om een toename van hyperreactiviteit voor histamine te induceren. Een dergelijke toename kon niet worden gerelateerd aan een late reactie in de neus.

In hoofdstuk 9 wordt het samengaan van allergie en nasale hyperreactiviteit geillustreerd aan de hand van een uitvoerig beschreven ziektegeval. Bij een vogelhouder met rhinitisklachten kon een IgE-gemedieerde allergie voor de allergenen van parkieten, kanaries en duiven worden aangetoond. Tevens werd bij deze patiënt een nasale hyperreactiviteit vastgesteld, zowel aan de hand van de anamnese als neusprovocatie met histamine.

In hoofdstuk 10 worden de resultaten van de diverse studies samengevat. Hierbij worden ook overeenkomsten en verschillen tussen nasale en bronchiale hyperreactiviteit besproken.

CONCLUSIES:

Een aantal conclusies kan als volgt worden samengevat:

- *DE MEETMETHODE:*

  Bij de bepaling van hyperreactiviteit van het neusslijmvlies voor histamine zijn alleen reflex-gemedieerde symptomen als niezen en secretie van belang. Rhinomanometrie kan hierbij achterwege worden gelaten. Vooralsnog is meting van nasale hyperreactiviteit bij patiënten met rhinitis niet zinvol bij de diagnostiek, gezien de overlapping van drempelwaarden tussen de groepen patiënten en gezonde personen.

- *DE ONDERZOCHTE PATIENTEN:*

  a. Niet-allergische rhinitis vasomotoria. Bij patiënten met dit ziektebeeld bestaat er een heterogeniciteit in de respons op histamine en methacholine. Dit kan verantwoordelijk zijn voor de verschillen in bevindingen, die door andere onderzoekers zijn gerapporteerd.

  b. Infectieuze rhinitis. Hierbij speelt nasale hyperreactiviteit geen rol.

  c. Allergische rhinitis. Bij patiënten met allergische rhinitis bestaat een samenhang tussen symptomatologie en de nasale hyperreactiviteit voor histamine. Deze hyperreactiviteit neemt toe onder invloed van allergeenexpositie. Dit betekent, dat het vervolgen van nasale hyperreactiviteit bij individuele patiënten wel bruikbaar kan zijn en wel tijdens of na therapeutische interventie, zoals het vermijden van allergeen, medicamenteuze behandeling en hyposensibilisatie. Voorts zou de nasale hyperreactiviteit voor histamine als maat voor expositie aan allergenen gebruikt kunnen worden. Tenslotte zou het wellicht mogelijk zijn om de reactie op allergeenextract te voorspellen aan de hand van metingen van nasale hyperreactiviteit voor histamine.

NAWOORD

Geen enkel proefschrift is het werk van één persoon. Ook dit boekje is tot stand gekomen door de krachtsinspanning van velen.

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Oek Phaff, allergoloog en Pim Dalinghaus, longarts hebben mij bijgestaan als paranimf.

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Neeltje Gerth van Wijk-Stork - beeldend kunstenares en ontwerpster, nu gespecialiseerd in grafische computer technieken - heeft mij geholpen met vormgeving en typografie. Ik ben haar erkentelijk hiervoor. Zij is mij dierbaar.

CURRICULUM VITAE

De schrijver van dit proefschrift is geboren op 12-10-1953 te Den Haag. Na voltooien van de middelbare school (gymnasium β) heeft hij tussen 1972 en 1981 geneeskunde gestudeerd aan de Universiteit van Amsterdam. In 1979-1980 was hij gedurende zes maanden werkzaam aan een onderzoek naar insektallergie op het Centraal Laboratorium van de bloedtransfusie dienst (dr. R.C. Aalberse) te Amsterdam. In 1981-1982 werd de militaire dienst vervuld als arts-assistent op de afdeling Inwendige Geneeskunde (dr. M. van Zoeren) van het Mil. Hospitaal "dr. A. Mathijssen" te Utrecht. In september 1982 werd de opleiding tot allergoloog aangevangen (opleider dr. P.H. Dieges). De opleiding werd gevolgd op de afdeling Allergologie (dr. P.H.Dieges), Inwendige Geneeskunde II (prof. JHP Wilson) en Longziekten (prof. dr. C. Hilvering) van het AZR-Dijkzigt te Rotterdam. Sinds september 1986 is hij werkzaam als staflid op de afdeling Allergologie van het AZR-Dijkzigt, en heeft hij een honoraire aanstelling als universitair docent bij het instituut Keel- ,Neus en Oorheelkunde van de Erasmus Universiteit te Rotterdam. In 1987 werd hij secretaris, sinds 1989 is hij voorzitter van het Concilium Allergologicum.

Colofon

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