Capsaicin in Idiopathic Rhinitis

A Hot Topic

J.B. van Rijswijk

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Jeroen Bernard van Rijswijk
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PROMOTIECOMMISSIE

Promotoren:	Prof. dr. C.D.A. Verwoerd Prof. dr. W.J. Fokkens
Overige leden:	Prof. dr. H.C. Hoogsteden Prof. dr. P.J. Koudstaal Prof. dr. P. van Cauwenberge
Copromotor:	Dr. H.M. Blom

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CHAPTER I

Introduction

Based on:

Idiopathic Rhinitis, the ongoing quest.

J.B. van Rijswijk, H.M. Blom and W.J. Fokkens

Allergy, 2004 in press

ABSTRACT

The term rhinitis in daily practice is used for nasal dysfunction causing symptoms like nasal itching, sneezing, rhinorrhea and or nasal blockage. Chronic rhinitis can roughly be classified into allergic, infectious or ideopathic rhinitis. When allergy, mechanical obstruction and infections have been excluded as the cause of rhinitis, a number of poorly defined nasal conditions of partly unknown aetiology and pathophysiology remain. The differential diagnosis of nonallergic noninfectious rhinitis is extensive.

Although the percentage of patients with nonallergic noninfectious rhinitis with a known cause has increased the last decades, still about 50% of the patients with nonallergic noninfectious rhinitis has to be classified as suffering from idiopathic rhinitis, or rather e causa ignota. Specific immunological, clinical and sometimes radiological and functional tests are required to distinguish known causes. Research to the underlying pathophysiology of idiopathic rhinitis has moved from autonomic neural dysbalans to inflammatory disorders (local allergy), the nonadrenergic noncholinergic sensory peptidergic neural system and central neural hyperesthesia, still without solid ground or proof. This review summarizes the currently known causes for nonallergic noninfectious rhinitis and possible treatments. Also possible pathophysiological mechanisms of idiopathic rhinitis are discussed.

INTRODUCTION

Rhinitis is a very common disorder known to all of us. Most people suffer from an infectious viral rhinitis, or common cold, at least once a year. It generally proofs to be a self limiting disease disappearing in 1 or 2 weeks without specific treatment. This in contrast to chronic rhinitis, affecting upto 20% of the general population (1).
Chronic rhinitis can be due to common factors such as allergy, smoking or less common factors such as xylometazoline abuse or cystic fibrosis. Rhinitis means inflammation of the nasal mucosal membrane. However, markers of inflammation are not examined in routine clinical work. Therefore, the term rhinitis in daily practice is used for nasal dysfunction causing symptoms like nasal itching, sneezing, rhinorrhea and or nasal blockage (1).

RHINITIS CLASSIFICATION

Chronic rhinitis can roughly be classified into allergic, infectious or nonallergic-noninfectious (Figure 1) (1-3). The exact figures are unknown but most ENT clinics report a 50-50 % division between allergic and nonallergic patients in perennial rhinitis (4).

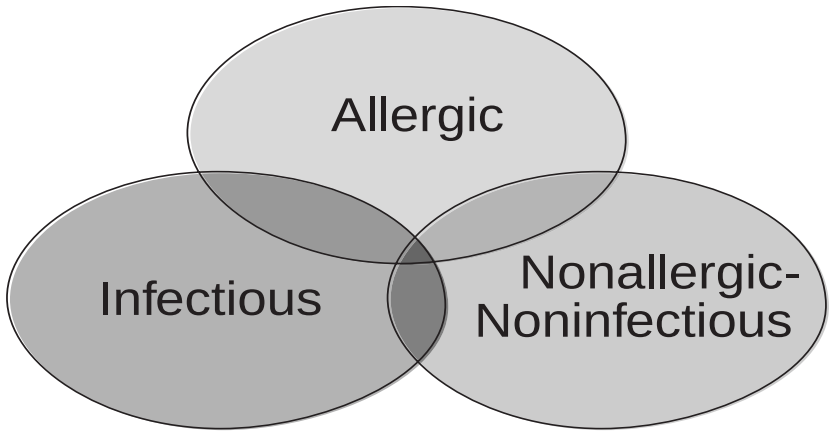


Figure 1. Schematic representation of allergic, infectious and nonallergic/noninfectious rhinitis.

Allergic rhinitis is clinically defined as a symptomatic disorder of the nose induced by an IgE-mediated inflammation after allergen exposure of the nasal mucosa. The diagnosis of allergy is based on diagnostic tests for allergy, like skin prick tests and or measurement of specific serum IgE.
The disease is nonallergic when allergy has not been proven by proper allergy examination (history, skin prick testing, measurement of serum specific IgE antibodies).
Rhinitis is called noninfectious when the persistent nasal discharge is clear and watery, and not purulent. Detection of micro-organisms (viruses, bacteria, fungi) is generally not used as a diagnostic criterion.
When allergy, mechanical obstruction and infections have been excluded as the cause of rhinitis, a number of poorly defined nasal conditions of partly unknown aetiology and pathophysiology remain. The differential diagnosis of nonallergic noninfectious rhinitis is extensive (5) (Table 1). The mechanisms are only partly unravelled. If the pathophysiology is unknown, the term idiopathic rhinitis (IR) is used.

Table 1. The differential diagnosis of nonallergic noninfectious rhinitis

Occupational (Irritant)
Drug induced:
Rhinitis Medicamentosa (topical vasoconstrictive α-adrenoceptor agonists)
Other drugs
Hormonal
Rhinitis of the Elderly
NARES
Smoking
Idiopathic Rhinitis (e causa ignota)

NONALLERGIC NONINFECTIOUS PERENNIAL RHINITIS

Nonallergic noninfectious perennial rhinitis can be divided in disorders with known and in disorders with unknown pathology.

Occupational nonallergic rhinitis

Occupational rhinitis arises in response to an air-borne agent present in the workplace. Many occupational agents are irritant and nonallergic hyper-responsiveness may occur.

Most occupational agents inducing nonallergic rhinitis are small molecular weight compounds such as isocyanates, aldehydes, ninhydrin, and pharmaceutical compounds (6, 7). More than 250 different chemical entities have been identified. Although these can act as reactive haptens, non-immunological mechanisms are common.

Some compounds, like chlorine, can induce irritant rhinitis in 30 to 50% of the exposed workers (8, 9).

Rhinitis Medicamentosa

Long-term use of topical nasal vasoconstrictors (like xylometazoline-hydrochloride and other α-adrenoceptor agonists) often results in rhinitis medicamentosa with possible histologic mucosa changes and drug addiction. Rhinitis medicamentosa can be defined as a condition of nasal hyperreactivity, mucosal swelling, rebound nasal congestion and tolerance that is induced, or aggravated, by the overuse of topical vasoconstrictors with or without a preservative (10). In the Netherlands this pathology is regularly seen in the second echelon since the over-the-counter availability of these medicines. Generally these patients can be adequately treated by lucid exposition, vasoconstrictor withdrawal and a topical corticosteroid spray to alleviate the withdrawal process (11). After a successful vasoconstrictor withdrawal a possible remaining nasal disorder (if any) can be treated.

Other drugs inducing nonallergic rhinitis

A range of medications is known to cause nasal symptoms. ACE inhibitors, reserpine, hydralazine, guanethidine, methyldopa, α-adrenoceptor antagonists such as prazosin and phentolomine , ß-blockers, immunosuppresives, oral contraceptives, aspirin, and other non-steroidal anti-inflammatory agents have all been associated with nasal symptoms, as have intra-ocular ophthalmic preparations (ß-blockers) (1, 12). Also psychotropic agents like thioridazine, chlordiazepoxide, chlorpromazine, perphenazine, amitriptyline, and alprazolam can have nasal side-effects (13) (Table 2).

Table 2. Drugs influencing nasal function

Medicin group:	*Examples:*
Nasal vasoconstrictors	*oxymetazoline, xylometazoline, ephedrine,*
ACE inhibitors	*benazepril, captopril, cilazapril, enalapril, fosinopril, lisinopril, perindopril, ramipril, quinapril, trandolapril*
Antihypertensiva	*guanfacine, reserpine, hydralazine, methyldopa, guanethidine,*
α- adrenoceptor antagonists	*prazosin, phentolomine*
β- blockers (also intra-ocular)	*carvedilol, propranolol, sotalol, tertatolol, timolol, alprenolol, oxprenolol, pindolol, atenolol, betaxolol, bisoprolol, esmolol, metoprolol, nebivolol, acebutolol, celiprolol*
Oral contraceptives	
Aspirin and other NSAID's	
Psychotropics	*chlorpromazine, thioridazine, chlordiazepoxide, amitriptyline, perphenazine, alprazolam*
Immunosuppresives	*cyclosporin, mycophenolic acid*

Hormonal rhinitis

Changes in the nose are known to occur during the menstrual cycle, puberty, pregnancy and in specific endocrine disorders such as hypothyroidism and acromegaly (14-16). Hormonal imbalance may also be responsible for the atrophic nasal change in post-menopausal women. A persistent hormonal rhinitis or rhino-sinusitis may develop during pregnancy in otherwise healthy women. Its severity parallels the blood oestrogen level (99). The symptoms usually quickly disappear after delivery.

Rhinitis of the Elderly

Rhinitis of the elderly, or senilic rhinitis as it is called in the Netherlands, is a characteristic clinical picture of the elderly patient suffering from a persistent clear rhinorrhea without nasal obstruction or other nasal symptoms. Patients often complain of the classical drop on the tip of the nose. The first treatment option is intranasal ipratropium bromid (up to 6 times a day) generally with a good clinical result, suggesting an overactivity of the parasympathetic neural system (17, 18).

Nonallergic Rhinitis with Eosinophilia Syndrome

The nonallergic rhinitis with eosinophilia syndrome (NARES) was originally described in 1981 by Jacobs (19). He described patients with perennial nasal symptoms of sneezing paroxysms, profuse watery rhinorrhea, and pruritus of the nasopharyngeal mucosa in an "on-again-off-again" symptomatic pattern with a profound eosinophilia in the nasal smear (a nasal smear with more than 25% eosinophils (20)) and no signs of allergy as tested by skin prick testing and measurement of total and specific IgE in the nasal secretion. Trigger factors associated by the patients with the acute onset of nasal symptoms were non or unknown in 42%, weather changes in 31%, odours in 15%, and noxious or irritating substances in 12%. The same sort of patient group, with perennial symptoms of nasal hyperreactivity involving sneezing, rhinorrhea, nasal obstruction, pruritus and frequent hyposmia was later described by others (21, 22). Moneret-Vautrin suggested that NARES is a precursor of aspirin sensitivity (21). Other groups were not able to find eosinophilia in their population of nonallergic rhinitis patients (23, 24). This

in contrast with a recent article of Powe describing nasal mucosa eosinophilia in IR patients comparable to perennial allergic rhinitis patients (25). He suggests that NARES is a local IgE mediated response (local allergy) which does not result in systemic Th2 responses. Although local IgE production has been made plausible already in the eighties by Platts-Mills and others (26), the final prove came recently. It has been proven by at least two groups now that local IgE production takes place in the nasal mucosa of allergic patients (27, 28). However it remains to be proven that the same mechanism also occurs in IR patients and whether this situation is stable over time or that these patients, like has been shown in small children, develop allergic rhinitis in due time.
The definition of NARES as a subgroup of nonallergic noninfectious rhinitis is relevant for therapy because they seem to respond well to nasal corticosteroids (29). This in contrast to some other subgroups of nonallergic noninfectious rhinitis.

Smoking Rhinitis
Smoke, in particular cigarette smoke, is known for its irritative effect on the mucosa of the respiratory tract. In passive smoking nonallergic children and in smoking adults a mucosal cellular infiltration with Th2 like profile including eosinophils, increased IgE+ cells and increased Il-4 is found (30-32). Because smoking results in many individuals in the same clinical picture of rhinitis with rhinorrhea and nasal obstruction it has to be viewed as a cause of rhinitis in its own right. It might even be that (part of the) NARES type of nonallergic rhinitis is caused by (passive) smoking inducing an 'allergy like' inflammatory response (30, 31, 33).

Idiopathic Rhinitis
If all the possible causes are excluded, a significant part of the nonallergic noninfectious rhinitis patients group persist. Syndromes of chronic rhinitis with an unknown aetiology were formerly referred to by us as NANIPER. Other terms like NINAR (noninfectious, nonallergic rhinitis) are also purely descriptive (34).
In accordance with the ''World Health Organisation Initiative, Allergic Rhinitis and its Impact on Asthma'' (ARIA) (1), we will be using the term idiopathic

rhinitis (IR) to describe this pathology. IR, formerly also called vasomotor rhinitis, is a diagnosis of exclusion and is given to patients suffering from perennial nasal congestion, rhinorrhea and or sneezing with no identifiable aetiology. IR is unrelated to allergy, infection, structural lesions, polyposis and other systemic diseases (as mentioned above).

IR, being a diagnosis per exclusionem, is solely diagnosed on patient complaints. The first question therefore may be whether this disease really exists. Occasional sneezing, rhinorrhea in the morning and upon exposure to cold and polluted air can be considered a normal nasal response. Some persons consider even slight nasal symptoms to be abnormal and seek medical advice for that reason. Inquiry about the hours with daily symptoms may help to make a distinction between a normal physiologic response and disease. Also the use of a daily record card (DRC, Table 3) to score symptom duration and intensity, possibly combined with peak nasal inspiratory flow measurements, can give the physician more insight in the severity of the disease. Marked

Table 3. Design of the daily record chart for defining nasal symptoms of IR patients.

Possible scores on the daily record chart	
Nasal blockage:	0 = absent
(not being able to breathe freely through the nose)	1 = between 0-1 h per half day
Clear nasal discharge: (runny nose)	2 = between 1-2 h per half day
	3 = more than 2 h per half day
Sneezing	0 = absent
Coughing	1 = less than 5 periods per half day
	2 = between 5-10 periods per half day
	3 = more than 10 periods per half day
Mucus production:	0 = absent
(yellow, green or brown)	1 = present

discrepancies between description of the problem at the first visit and data from these daily measurements can be found (35).

DIAGNOSTIC CRITERIA FOR IDIOPATHIC RHINITIS

To exclude all known prevailing causes of chronic rhinitis one should at least take a proper history (medication, smoking in previous 6 months, occupation, etc.), adequately exclude commonly occurring inhalation allergies (skin prick test and or specific serum IgE measurement) and perform rhinoscopia anterior and nasendoscopy to exclude gross anatomical aberrations and nasal polyps.
The mucosa of the nose and sinus are contiguous and thus chronic nasal complaints can also be induced by a (accompanying) chronic sinusitis. When in doubt of a possible chronic sinusitis one should not hesitate to perform CT-scan imaging. However, if the history and the nasendoscopy lack criteria pointing at possible sinus problems, CT-scan imaging is, in our opinion, not obligatory for diagnosing IR.

Nasal complaints as a IR selection-criterion
After having excluded all known causes of chronic rhinitis one is left with a group of patients with nasal complaints of unknown pathology (IR) (Table 4). This means that the studied patient group is probably a melting pot of patients suffering from nasal complaints, with presumably variable pathogenesis. To study, select and define a group of patients, and more, measure the effects of interventions, positive criteria are needed to make the group as homogeneous as possible. As IR is solely diagnosed on patients complaints we use (and have used in all our previous studies to IR) a daily record chart (Table 3) on which patients have to reach a minimum symptom score to be classified as IR patient. The minimum is set, using the basis of the definition of rhinitis put forward by Mygind and Weeke (36). In affected patients, periods of nasal discharge, sneezing and / or congestion have to persist for an average of at least 1-hour per day on at least five days during a period of fourteen days.

Table 4. Exclusion criteria for IR

Positive allergy test (specific serum IgE, skin prick test, etc.)
Smoking (in the previous 6 months)
Nasal polyps or a history of nasal polyps.
Significant anatomical abnormalities affecting nasal function.
Nasal or paranasal sinus infection (abnormal sinus X-ray).
Pregnancy or lactation
Inability of the patient to stop taking medication affecting nasal function.
Beneficial effect of nasal corticosteroid spray (probably NARES patient)

Nasal hyperreactivity as a IR selection-criterion

Various stimuli have been used to try to discriminate IR patients from normal controls. Nasal hyperreactivity to non-specific stimuli is a common and characteristic feature of patients with chronic rhinitis. Hyperreactivity only describes the increased reactivity of the nasal mucosa to 'nonspecific'stimuli such as smoke, strong odors and other irritants but does not point to any cause of the disease. In addition, patients with allergic rhinitis usually complain of hyperreactivity to nonallergic stimuli, obviously as a direct result of allergic inflammation. Until now, the most common diagnostic test for measuring nasal hyperreactivity was intranasal histamine provocation (37). Histamine provocations in allergic rhinitis and asthma are proven to be a good test for hyperreactivity.

Histamine provocation, however, fails to differentiate between patients with IR and control subjects (38, 39). It has been shown that methacholine is able to discriminate IR patients with persistent rhinorrhea from controls but not IR patients with blockage as their main symptom (37). Also IR patients cannot be characterised by increased responsiveness to capsaicin provocation(40).

Cold dry air (CDA) provocation as an effective tool in quantifying the secretory response of hyperreactivity in persons susceptible to CDA was first published by the Baltimore group (38, 41). The Rotterdam group subsequently proposed a new standardised intranasal CDA provocation method, which is able to make a reasonable distinction between IR patients and controls (39). This new standardised intranasal CDA provocation resulted in increased mucus production and nasal blockage in a dose-dependent manner in patients

with IR but not in control subjects. Sneezing did not occur. The reproducibility, sensitivity and specificity of this CDA provocation gives us a useful diagnostic tool in IR patients and the possibility to monitor treatment effect (39).

CONSIDERATIONS ON POSSIBLE PATHOPHYSIOLOGIC MECHANISMS

In spite of trying to form an IR patient group as homogenous and uniform as possible it still has to be anticipated that IR is a cumulation of different pathophysiological entities. With the limited data available at the moment, we will speculate which pathophysiological mechanisms might play a role in IR. Whether the roles of these mechanisms are major or minor and which are important for many or few patients with IR has to be further elucidated.

Chronic inflammatory disorder

The proposed pathophysiological mechanisms for IR include a chronic inflammatory disorder of antigenic (local allergy) or neurogenic nature (42-45). A pivotal characteristic in the pathophysiological concept of inflammation is an influx of inflammatory cells in the affected tissue. In symptomatic allergic rhinitis patients, an increase of inflammatory cells has been observed in the nasal mucosa and this increase is positively correlated to nasal complaints (46-48).

In a nasal biopsy study (35) we did not found any significant difference for nasal mucosal lymphocytes, antigen-presenting cells, eosinophils, macrophages, monocytes, mast cells and other IgE-positive cells between IR patients and controls. This contrasts with a recent study of Powe et al. who found significantly more nasal mucosa mast cells and eosinophils in a group of IR (and allergic rhinitis) patients compared to a group of normal individuals (25). They examined whole, full-length, full-thickness concha inferior specimens resected under general anaesthesia.

The difference in study outcome may be explained by a more severe pathology in the IR group of Powe et al. warranting total turbinectomy. Another explanation could be the difference in biopsy size (average surface area of 1.6 mm^2 in our studies). Nasal cellular infiltrates show a focal localisation of cell populations which can be better averaged in larger biopsies.

It may also be the case that our IR patient group contains significantly fewer NARES patients (2 of the 65) compared to the patient group studied by Powe et al. The reason for this could be the fact that a Dutch rhinitis patient will not be sent to the ENT department before being treated with local corticosteroids by his or her general practitioner (49). In addition, as might be expected, it seems that NARES patients and or patients with an occult local allergy form an IR subgroup which responds well to nasal corticosteroids (29).
In two other studies, we failed to ascertain a relation between the number of immunocompetent cells and nasal complaints in IR patients (23, 50). A significant reduction of immunocompetent cells in the nasal mucosa of IR patients treated with nasal steroids (fluticasone aqueous nasal spray) was not accompanied by a reduction in nasal complaints (23) and, inversely, a significant reduction in nasal complaints in a group of IR patients treated with topical capsaicin aqueous nasal spray was not accompanied by a reduction in the numbers of inflammatory cells (50).
In a placebo controlled study Gerth van Wijk did not find a therapeutic effect for capsaicin in a group of perennial allergic rhinitis patients allergic to house dust mite (51). The capsaicin treatment protocol in this study was identical to the one used by Blom showing a significant and long-term reduction of symptoms in a group of IR patients (50). It was speculated that allergic rhinitis was not affected by capsaicin through domination of nasal inflammation, whereas the efficacy of capsaicin in IR may be due to domination of the peptidergic system in the absence of nasal inflammation (51).

Neurogenic mechanisms
The neural regulation of the upper airways is complex and consists of a number of interacting nervous systems. Sensory, parasympathetic and sympathetic nerves regulate epithelial, vascular and glandular processes in the nasal mucosa. The anatomically defined sensory parasympathetic and sympathetic neural systems contain heterogeneous populations of nerve fibres often containing unique combinations of neurotransmitters and neuropeptides (34, 52).

Parasympathetic / sympathetic neural dysbalans

In 1959 Malcomson stated that IR was caused by an autonomic dysbalans (53). Normally, base line sympathetic tone provides a constant alpha and beta adrenergic receptor stimulation (54). The marked alpha-1 predominance in nasal blood vessels leads to vasoconstriction (55). Underactivity of the sympathetic nervous system leads to nasal obstruction (56). Parasympathetic effects on blood vessels are minimal under basal conditions. Stimulation of cholinergic nerves leads to hypersecretion and dilatation of mainly resistance vessels (increase in nasal blood flow) and to some extent capacitance vessels (decrease in nasal patency). Overactivity of the parasympathetic system leads to rhinorrhea (56).

However, van Megen, in a group of 4 patients, was unable to show significant differences in alpha-2, alpha-1 and beta-adrenoreceptors between controls and vasomotor rhinitis patients (57).

On the other hand some data suggesting a sympathetic involvement in IR has recently been published by the Liverpool group. Although the magnitude between patients with IR and controls were small, patients with IR were found to have an abnormal nasal response compared to controls after isometric exercise (58) and after axiallary pressure (59). The specificity of these findings compared to other forms of rhinitis however has to be confirmed.

Non-adrenergic non-cholinergic or peptidergic neural system

Due to extensive research in the seventies and eighties it was discovered that perivascular and intra-epithelial nonadrenergic noncholinergic (NANC), sensory nerve fibres contain neuropeptides (including VIP, substance P (SP), calcitonin gene related peptide (CGRP), id.) which were demonstrated in the nasal mucosa of various mammals including man (60, 61). The actions of these neuropeptides are limited by degradation by neutral endopeptidase (62). These neuropeptides are locally released from peptidergic neurons (antidromic release), mainly unmyelinated sensory C-fibres, in the nasal mucosa after activation by unspecific stimuli, and can be responsible for the symptoms of IR (63-65). Stimulation can be induced by inflammatory mediators, like histamine and bradykinin but also by a number of inhaled irritants like nicotine, cigarette smoke, formaldehyde and capsaicin (66-68).

The unmyelinated sensory c-fibres or ‘pain receptors’ are specifically sensi-

tive to capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent agent of hot red pepper (69, 70). Nasal capsaicin provocation results in rhinorrhea, nasal blockage and sneezing (52). This sensory neural stimulation may produce these effects either through an orthodromic, central neural reflex, associated with efferent, predominantly parasympathetic, neurotransmission, and or via an anti-dromic, afferent, local release of neuropeptides from sensory neurons (71) (Figure 2). Repeated applications of capsaicin, however, lead to desensitisation and even degeneration of peptidergic unmyelinated sensory C-fibres (72, 73).

Therefore the hypothesis, suggested among others by Wolf (74), that a hyperactive non-adrenergic non-cholinergic peptidergic neural system is the underlying pathophysiology in IR, may offer an explanation for the beneficial effect of intranasal capsaicin with these patients.

This hypothesis was corroborated by Lacroix, who reported an increased concentration of neuropeptides in a group of chronic IR patients (65), improvement of symptoms by local treatment of capsaicin giving a 50% reduction in CGRP-Li content in nasal biopsies (75), and a correlation between symptom intensity and CGRP-Li concentration in nasal mucosa (76). Several studies have been published showing a therapeutic effect in IR patients for repeated topical applications of capsaicin (77-79).

However, the mechanism explaining this therapeutic effect remains for the greater part unclear. In spite of the CGRP-Li reduction found by Lacroix we did not find any significant difference in pan-neurogenic staining of nasal mucosa using neurofilament and synaptophysine between capsaicin and placebo treated patients 2 weeks, 3 months and 9 months after therapy although there was a significant therapeutic effect measured with visual analogue scale (VAS) (50). Also Wolf was unable to show a reduction of NANC-fibres in the nasal mucosa in IR patients after successful capsaicin treatment (77). He suggested capsaicin receptor blockage as a possible explanation for the capsaicin treatment effect. Although sounding attractive it seems improbable that capsaicin receptor blockage alone can result in the long lasting therapeutic effect observed in IR patients.

These findings, however, do not discard the hypothesis of a hyperactive non-adrenergic non-cholinergic peptidergic system, as the activity of this system was not measured. A functional hyperactivity of this system, not captured by

histological changes, could still be the underlying pathophysiological process in IR.

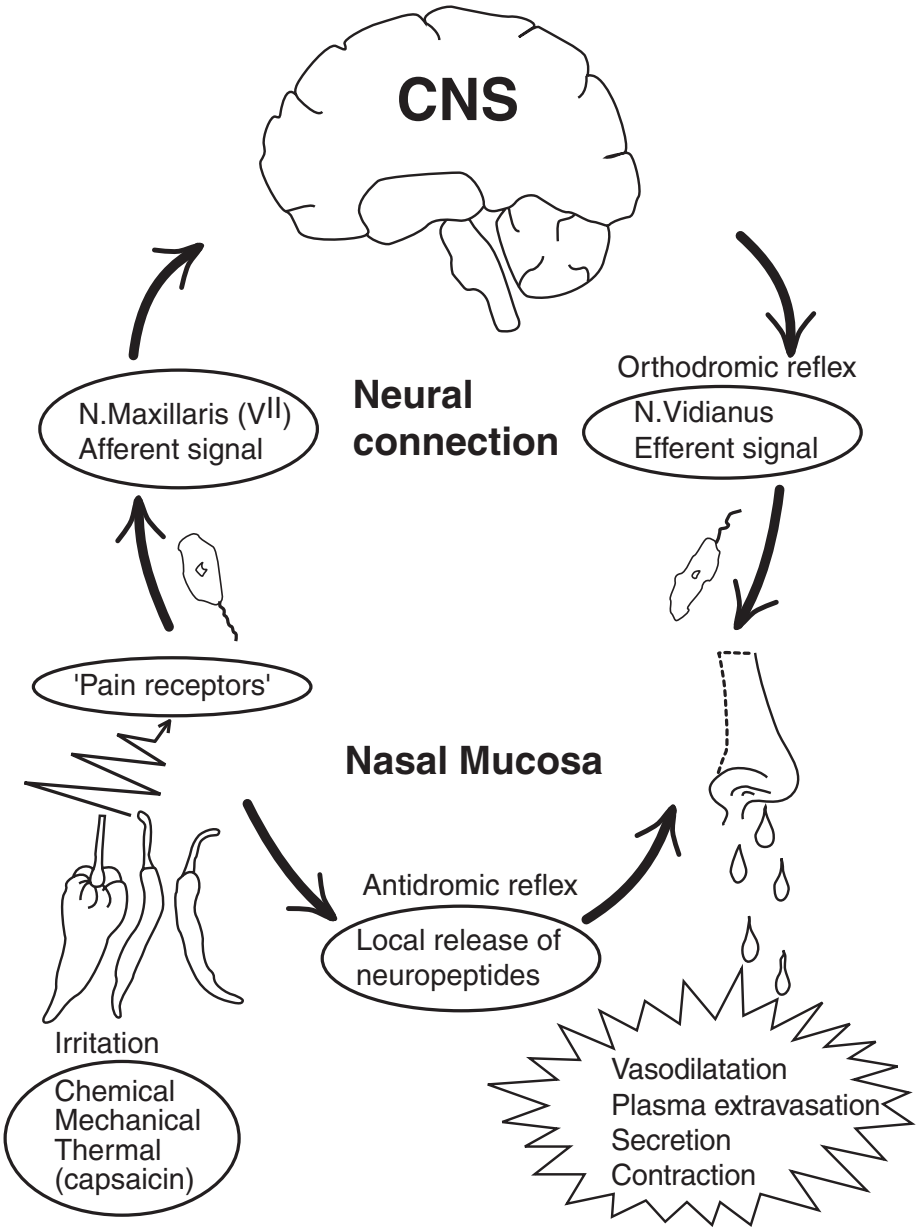
Hyper- or dysesthesia at the CNS level

Another possibility, raised by Sanico, is a hyper- or dysesthesia at the central nervous system (CNS) level as an explanation for IR (34) (Figure 2). This would explain the lack of changes/differences in cell counts and neurogenic staining in the several studies mentioned above. According to this theory a functional or numerical downregulation of the unmyelinated peptidergic sensory C-fibres would also explain the therapeutic effect of intranasal capsaicin application (34). One might speculate that CNS hyperesthesia is induced by a vicious circle of environmental irritants and changes in atmospheric conditions, perceived as an ever irritating stimulans at the CNS level, and the protective responses hereupon like rhinorrhea, vascular congestion and sneezing. Although sounding attractive, it will be hardly impossible to prove due to the key role allocated to the CNS in this theory.

Nitric Oxide synthase

Recently Ruffoli, et al. reported a strong localisation of nitric oxide synthase (using NADPH-diaphorase cytochemistry) in the vascular smooth muscle cells of the cavernous sinuses in 7 IR patients compared to the nitric oxide synthase localisation in unaffected subjects (80). In their article they hypothesise that local, anti-dromic neuropeptide release of sensory fibres in the nasal mucosa of IR patients could cause nitric oxide synthase induction in vascular smooth muscle cells through a c-AMP dependent mechanism, giving nasal congestion. However, no significant differences were found in endothelial nitric oxide synthase localisation between IR patients and

Figure 2. Simplified scheme of autonomic and peptidergic innervation of the nasal mucosa. Irritation initiates an afferent, sensory signal. After central processing this will lead to an efferent, predominantly parasympathetic signal giving rise to increased secretion and vasodilatation: the orthodromic reflex. The initial irritation also induces the local release of neuropeptides (SP, CGRP, etc.) from sensory nerves in the nasal mucosa also resulting in increased vasodilatation, vascular permeability and secretion: the antidromic reflex.


CNS
Neural connection
N.Maxillaris (VII)
Afferent signal
Orthodromic reflex
N.Vidianus
Efferent signal
'Pain receptors'
Nasal Mucosa
Antidromic reflex
Local release of neuropeptides
Irritation
Chemical
Mechanical
Thermal
(capsaicin)
Vasodilatation
Plasma extravasation
Secretion
Contraction

unaffected subjects. Although interesting, it is to early for definitive conclusions about a possible role for nitric oxide synthase in the pathophysiology of IR.

TREATMENT MODALITIES

In general one can state that the less is known about a disease (and its underlying pathophysiology) the more treatment options there are available. This in particular counts for IR with a wide range of available therapies, surgical as well as pharmacotherapeutical, all claiming partial success. With the exception of rhinitis of the elderly where ipratropium bromid is the obvious first treatment of choice (see above), there is no obvious best treatment or first treatment to start with in nonallergic noninfectious rhinitis.

Topical or systemic sympathicomimetica

A topical sympathicomimeticum provides instant relief but only for a short period. It should not be used for more than 1 week in view of the risk for developing rhinitis medicamentosa (see above). Considering this it only has a very limited role in the therapeutic arsenal of chronic IR. Systemic sympathicomimetica, although widely used in some countries, seem to have many considerable side effects (81).

Topical steroids

In our view, a topical steroid aqueous spray once or bi daily, preferably combined with nasal 0.9% saline douches, is the treatment of first choice in IR (82). It should be tried for a minimum period of 6 weeks before treatment evaluation should take place, for it can take a few weeks to reach the maximum treatment effect (83, 84). Often IR patients, referred by the general practitioner to the second or third echelon due to treatment failure after short time use of a topical steroid spray, react as yet favourable to topical steroid spray using it for a longer period.

However, with the exception of NARES patients who in general respond well to a topical steroid spray (see above) we feel that for the rest of the nonallergic noninfectious rhinitis patients topical steroids often do not provide the same relief as they do in allergic rhinitis (23, 43).

Antihistamines

Sneezing as a predominant complaint in nonallergic noninfectious rhinitis is rare, but if present antihistamines can be prescribed, sometimes with good results (1). Also in the case of extreme hyperreactivity antihistamines are sometimes helpful, possibly because of a pathophysiologic role for mastcell degranulation releasing histamine in these patients. Two double-blind placebo controlled trials have been published showing a therapeutic effect for azelastine nasal spray in IR patients with nasal obstruction and or rhinorrhea (85, 86). The precise mode of action (antihistaminic, antiinflammation, or otherwise) remains to be elucidated (87).

The older antihistamines often also have some anticholinergic action possibly contributing to the therapeutic effect.

Ipratropium bromid

Ipratropium bromid is an anticholinergic drug used mainly in the treatment of asthma. Clinical studies using this drug as a nasal spray have shown it to be effective in reducing the severity and duration of the rhinorrhea in nonallergic noninfectious rhinitis (17, 88). It is therefore the first treatment option in rhinitis of the elderly (see above).

Capsaicin

As mentioned before several studies have been published showing a therapeutic effect in IR patients for repeated topical applications of capsaicin (77-79). Although direct observations explaining the efficacy and working mechanisms of capsaicin are lacking, it is the therapy of choice in IR patients in our institutes when a minimum period of 6 weeks of treatment with a topical steroid spray has proven unbeneficial in relieving symptoms. Of course, as with other therapies for IR, not all patients will be cured but a great percentage of patients (we feel circa 75%) will show a long lasting (more than one year) relieve of symptoms. When IR symptoms return after a symptom free period upon capsaicin therapy it is very worthwhile to treat these patients (after careful examination excluding again all known causes of rhinitis) for a second (or sometimes third) time with capsaicin for the favourable reaction to capsaicin will most probably repeat itself (unpublished data). This was also reported by Wolf (77).

Surgery

Most authors feel that surgical therapy should only be considered for those patients who fail to obtain symptomatic relief with medical therapy (20, 56, 82). Surgical procedures for nonallergic noninfectious rhinitis aim to either modify the size of the inferior turbinate or to denervate the nose of its autonomic supply. Turbinate reduction can be a valuable alternative when medical therapy fails. The surgical scalpel, chemical sclerosing solutions, electrocautery, cryosurgery, snake venom and laser surgery have all been reported to diminish obstruction complaints (89-93). The duration of effectiveness reported varies from 6 months to several years (55). Golding-Wood described the effect of vidian neurectomy (94, 95). This procedure is effective in relieving excessive secretion but not so much the obstruction. Both preganglionic parasympathetic and sympathetic fibres are interrupted. Grote concluded that vidian neurectomy was not the panacea it was claimed to be, since renervation would occur (96). This was corroborated by several authors (97, 98).

CONCLUSION

Although the percentage of patients with nonallergic noninfectious rhinitis with a known cause has increased the last decades, still about 50% of the patients with a nonallergic noninfectious rhinitis have to be classified as suffering from IR. Specific immunological, clinical and sometimes radiological and functional tests are required to distinguish known causes. Research to the underlying pathophysiology of IR has moved from autonomic neural dysbalans to inflammatory disorders (local allergy), the NANC peptidergic neural system (with or without nitric oxide synthase induction in vascular smooth muscle cells) and central neural hyperesthesia, still without convincing solid ground or proof. It can be expected that in the next future some more explanatory pathophysiologic mechanisms for nonallergic noninfectious rhinitis will be found, doing justice to the idea that the diagnosis IR is still a ‘melting pot’ of several pathophysiological conditions. Hopefully the future unravelling of this intriguing disease will lead to more specific and may-be better treatment options.

References

1. Bousquet J, van Cauwenberge P, Khaltaev N. Allergic Rhinitis and its Impact on Asthma (ARIA). J Allergy Clin Immunol 2001;108(5 part 2):S147-334.
2. Group IRMW. International Consensus Report on the diagnosis and management of rhinitis. Allergy 1994;49(19 Suppl):S1-34.
3. Dykewicz MS, Fineman S, Skoner DP, Nicklas R, Lee R, Blessing-Moore J, et al. Diagnosis and management of rhinitis: complete guidelines of the Joint Task Force on Practice Parameters in Allergy, Asthma and Immunology. American Academy of Allergy, Asthma, and Immunology. Ann Allergy Asthma Immunol 1998;81(5 Pt 2):478-518.
4. Fokkens WJ. Thoughts on the pathophysiology of nonallergic rhinitis. Current Allergy and Asthma Reports 2002;2:203-209.
5. Bachert C. Persistent rhinitis - allergic or nonallergic? Allergy 2004;59 (Suppl. 76):11-15.
6. Slavin RG. Occupational rhinitis. Ann Allergy Asthma Immunol 1999;83(6 pt 2):597-601.
7. Saria A. Neuroimmune interactions in the airways: implications for asthma, allergy, and other inflammatory airway diseases. Brain Behav Immun 1988;2(4):318-21.
8. Leroyer C, Malo JL, Girard D, Dufour JG, Gautrin D. Chronic rhinitis in workers at risk of reactive airways dysfunction syndrome due to exposure to chlorine. Occup Environ Med 1999;56(5):334-338.
9. Shusterman D, Balmes J. Measurement of nasal irritant sensitivity to pulsed carbon dioxide: a pilot study. Arch Environ Health 1997;52(5):334-340.
10. Graf P. Long-term use of oxy- and xylometazoline nasal sprays induces rebound swelling, tolerance, and nasal hyperreactivity. Rhinology 1996;34(1):9-13.
11. Graf P. Rhinitis medicamentosa: aspects of pathophysiology and treatment. Allergy 1997;52(40 Suppl):28-34.
12. Kaufman HS. Timolol-induced vasomotor rhinitis: a new iatrogenic syndrome [letter]. Arch Ophthalmol 1986;104(7):970.
13. Mabry RL. Nasal stuffiness due to systemic medications. Otolaryngol Head Neck Surg 1982;91:93-94.
14. Incaudo G, Schatz M. Rhinosinusitis associated with endocrin conditions: hypothyroidism and pregnancy. In: M. Schatz, G. A. Settipane, ed. Nasal manifestations of systemic diseases: Providence, RI; 1991.
15. Ellegard E, Karlsson G. Nasal congestion during pregnancy. Clin Otolaryngol 1999;24:307-311.

16. Navarrete-Palacios E, Hudson R, Reyes-Guerrero G, Guevara-Guzman R. Correlation between cytological characteristics of the nasal epithelium and the menstrual cycle. Arch Otolaryngol Head Neck Surg 2003;129:460-463.
17. Malmberg H, Grahne B, Holopainen E, Binder E. Ipratropium (Atrovent) in the treatment of vasomotor rhinitis of elderly patients. Clin Otolaryngol 1983;8(4):273-6.
18. Dockhorn R, Aaronson D, Bronsky E, Chervinsky P, Cohen R, Ehtessabian R, et al. Ipratropium bromid nasal spray 0.03% and beclomethasone nasal spray alone and in combination for the treatment of rhinorrhea in perennial rhinitis. Ann Allergy Asthma Immunol 1999;82(4):349-359.
19. Jacobs RL, Freedman PM, Boswell RN. Nonallergic rhinitis with eosinophilia (NARES syndrome). Clinical and immunologic presentation. J Allergy Clin Immunol 1981;67(4):253-262.
20. Mikaelian AJ. Vasomotor rhinitis. [Review]. Ear Nose Throat J 1989;68(3):207-10.
21. Moneret-Vautrin DA, Hsieh V, Wayoff M, Guyot JL, Mouton C, Maria Y. Nonallergic rhinitis with eosinophilia syndrome a precursor of the triad: nasal polyposis, intrinsic asthma, and intolerance to aspirin. Ann Allergy 1990;64(6):513-8.
22. Ingels K, Durdurez JP, Cuvelier C, van Cauwenberge P. Nasal biopsy is superior to nasal smear for finding eosinophils in nonallergic rhinitis. Allergy 1997;52(3):338-341.
23. Blom HM, Godthelp T, Fokkens WF, Kleinjan A, Mulder PGM, Rijntjes E. The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with a non-allergic non-infectious perennial rhinitis. J Allergy Clin Immunol 1997;100(..):739-47.
24. Numata T, Konno A, Hasegawa S, Hanazawa T, Nagata H, Motosugi H, et al. Pathophysiological features of the nasal mucosa in patients with idiopathic rhinitis compared to allergic rhinitis. Int Arch Allergy Immunol 1999;119(4):304-313.
25. Powe DG, Huskisson RS, Carney AS, Jenkins D, Jones NS. Evidence for an inflammatory pathophysiology in idiopathic rhinitis. Clin Exp Allergy 2001;31(6):864-72.
26. Platts-Mills TA. Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. J Immunol 1979;122(6):2218-2225.
27. Kleinjan A, Vinke JG, Severijnen LW, Fokkens WJ. Local production and detection of (specific) IgE in nasal B-cells and plasma cells of allergic rhinitis patients. Eur Respir J 2000;15(3):491-497.
28. Durham SR, Gould HJ, Thienes CP, Jacobson MR, Masuyama K, Rak S, et al.

Expression of epsilon germ-line gene transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. Eur J Immunol 1997;27(11):2899-2906.
29. Small P, Black M, Frenkiel S. Effects of treatment with beclomethasone dipropionate in subpopulations of perennial rhinitis patients. J Allergy Clin Immunol 1982;70(3):178-82.
30. Vinke JG, Kleinjan A, Severijnen LW, Fokkens WJ. Passive smoking causes an 'allergic' cell infiltrate in the nasal mucosa of non-atopic children. Int J Pediatr Otorhinolaryngol 1999;51(2):73-81.
31. Villar MT, Holgate ST. IgE, smoking and lung function. Clin Exp Allergy 1995;25(3):206-209.
32. Rennard SI, Umino T, Millatmal T, Daughton DM, al. e. Evaluation of subclinical respiratory tract inflammation in heavy smokers who switch to a cigarette-like nicotine delivery device that primarily heats tobacco. Nicotine Tob Res 2002;4(4):467-476.
33. Bouwhuijsen ECA, Dijkstra MD, Severijnen LW, Kleinjan A, Fokkens WJ. Effects of ciagarette smoking on nasal mucosa inflammation. Allergologie 1996;19:56.
34. Sanico A, Togias A. Noninfectious, nonallergic rhinitis (NINAR): considerations on possible mechanisms. Am J Rhinol 1998;12(1):65-72.
35. Blom HM, Godthelp T, Fokkens WJ, Kleinjan A, Holm AF, Vroom TM, et al. Mast cells, eosinophils and IgE-positive cells in the nasal mucosa of patients with vasomotor rhinitis. An immunohistochemical study. Eur Arch Otorhinolaryngol Suppl 1995;1(9):S33-9.
36. Mygind N, Weeke B. Allergic and vasomotor rhinitis: Clinical aspects. Copenhagen: Munksgaard; 1985.
37. Gerth van Wijk R, Dieges PH. Nasal reactivity to histamine and methacholine: two different forms of upper airway responsiveness. Rhinology 1994;32:119-122.
38. Togias A, Lykens K, Kagey-Sobotka A, Eggleston PA, Proud D, Lichtenstein LM, et al. Studies on the relationships between sensitivity to cold, dry air, hyperosmolal solutions, and histamine in the adult nose. Am Rev Respir Dis 1990;141(6):1428-33.
39. Braat JP, Mulder PG, Fokkens WJ, Gerth van Wijk R, Rijntjes E. Intranasal cold dry air is superior to histamine challenge in determining the presence and degree of nasal hyperreactivity in nonallergic noninfectious perennial rhinitis. Am J Respir Crit Care Med 1998;157(6 Pt 1):1748-55.
40. Sanico AM, Philip G, Proud D, Naclerio RM, Togias A. Comparison of nasal mucosal responsiveness to neuronal stimulation in nonallergic and allergic rhinitis:

effects of capsaicin nasal challenge. Clin Exp Allergy 1998;28(1):92-100.
41. Togias AG, Naclerio RM, Proud D, al. e. Nasal challenge with cold, dry air results in release of inflammatory mediators. Possible mast cell involvement. J Clin Invest 1985;76:1375-1381.
42. Shatkin JS, Delsupehe KG, Thisted RA, Corey JP. Mucosal allergy in the absence of systemic allergy in nasal polyposis and rhinitis: a meta-analysis. Otolaryngol Head Neck Surg 1994;111(5):553-6.
43. Philip G, Togias AG. Nonallergic rhinitis. Pathophysiology and models for study. Eur Arch Otorhinolaryngol Suppl 1995;1(3):S27-32.
44. Carney AS, Jones NS. Idiopathic rhinitis: idiopathic or not? Clin Otolaryngol 1996;21(3):198-202.
45. Garay R. Mechanisms of vasomotor rhinitis. Allergy 2004;59 (Suppl. 76):4-10.
46. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. J Allergy Clin Immunol 1992;89(4):877-83.
47. Fokkens WJ, Holm AF, Rijntjes E, Mulder PG, Vroom TM. Characterization and quantification of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. Int Arch Allergy Appl Immunol 1990;93(1):66-72.
48. Braunstahl GJ, Overbeek SE, Fokkens WJ, Kleinjan A, McEuen AR, Walls AF, et al. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. Am J Respir Crit Care Med 2001;164(5):858-65.
49. Lundblad L, Sipila P, Farstad T, Drozdziewicz D. Mometasone furoate nasal spray in the treatment of perennial non-allergic rhinitis: a nordic, multicenter, randomized, double-blind, placebo-controlled study. Acta Otolaryngol 2001;121(4):505-9.
50. Blom HM, Severijnen LA, van Rijswijk JB, Mulder PG, Gerth van Wijk R, Fokkens WJ. The long-term effects of capsaicin aqueous spray on the nasal mucosa. Clin Exp Allergy 1998;28(11):1351-8.
51. Gerth van Wijk R, Terreehorst IT, Mulder PG, Garrelds IM, Blom HM, Popering S. Intranasal capsaicin is lacking therapeutic effect in perennial allergic rhinitis to house dust mite. A placebo-controlled study. Clin Exp Allergy 2000;30(12):1792-8.
52. Baraniuk JN. Sensory, parasympathetic, and sympathetic neural influences in the nasal mucosa. J Allergy Clin Immunol 1992;90(6 Pt 2):1045-50.
53. Malcomson KG. The vasomotor activities of the nasal mucous membrane. J

Laryngol 1959;73:73-75.
54. Lung MA. The role of the autonomic nerves in the control of nasal circulation. Biol Signals 1995;4(3):179-185.
55. Kimmelman CP, Ali GH. Vasomotor rhinitis. Otolaryngol Clin North Am 1986;19(1):65-71.
56. Jones AS, Lancer JM. Vasomotor rhinitis [editorial]. Br Med J 1987;294(6586):1505-6.
57. Megen van YJB. Neuroreceptors in nasal allergy . Nijmegen: University of Nijmegen; 1989.
58. Wilde AD, Cook JA, Jones AS. The nasal response to isometric exercise in non-eosinophilic intrinsic rhinitis. Clin Otolaryngol 1996;21(1):84-6.
59. Wilde AD, Jones AS. The nasal response to axillary pressure. Clin Otolaryngol 1996;21(5):442-4.
60. Lundblad L, Lundberg JM, Brodin E, Anggard A. Origin and distribution of capsaicin-sensitive substance-P-immunoreactive nerves in the nasal mucosa. Acta Otolaryngol 1983;96:485-493.
61. Uddman R, Alumets J, Densert O, Hakansson R, Sundler F. Occurence and distrbution of VIP nerves in the nasal mucosa and the tracheobronchial wall. Acta Otolaryngol 1978;85:443-448.
62. Baraniuk JN. Neuropeptides. Am J Rhinol 1998;12:9-16.
63. Baraniuk JN, Kaliner MA. Neuropeptides and nasal secretion. J Allergy Clin Immunol 1990;86(4 Pt 2):620-7.
64. Fang SY, Shen CL. Neuropeptide innervation and neuroendocrine cells in allergic rhinitis and chronic hypertrophic rhinitis. Clin Exp Allergy 1998;28(2):228-32.
65. Lacroix JS, Kurt AM, Pochon N, Bretton C, Lunberg JM, Deshusses J. Neutral endopeptidase activity and concentration of sensory neuropeptide in the human nasal mucosa. Eur Arch Otorhinolaryngol 1995;252:465-468.
66. Svensson C, Andersson M, Greiff L, Persson CG. Nasal mucosal endorgan hyperresponsiveness. Am J Rhinol 1998;12(1):37-43.
67. Anggard A. Basic mechanisms in autonomic nervous responses in specific and nonspecific nasal hyperreactivity. Acta Otolaryngol 1993;113:394-396.
68. Sanico AM, Atsuta S, Proud D, Togias A. Dose-dependent effects of capsaicin nasal challenge: in vivo evidence of human airway neurogenic inflammation. J Allergy Clin Immunol 1997;100(5):632-41.
69. Jancso N, Jancso-Gabor A, Szolcsanyi J. Direct evidence of neurogenic inflammation and its prevention by denervation and pretreatment with capsaicin. Br J Pharmacol Chemotherapy 1967(31):138-51.
70. Holzer P. Local effector functions of capsaicin sensitive nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other

neuropeptides. Neuroscience 1988;24:739-768.
71. Lundblad L, Saria A, Lundberg JM, Anggard A. Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons. Acta Otolaryngol 1983;96(5-6):479-84.
72. Holzer P. Capsaicin;cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 1991;43(2):143-201.
73. Kitajiri M, Kubo N, Ikeda H, Sato K, Kumazawa T. Effects of topical capsaicin on autonomic nerves in experimentally-induced nasal hypersensitivity. An immunocytochemical study. Acta Otolaryngol Suppl 1993;500(2):88-91.
74. Wolf G. [New aspects in the pathogenesis and therapy of hyperreflexive rhinopathy]. Laryngol Rhinol Otol (Stuttg) 1988;67(9):438-45.
75. Lacroix JS, Buvelot JM, Polla BS, Lundberg JM. Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy 1991;21(5):595-600.
76. Lacroix JS, Pochon N, Lundberg JM. Increased concentration of sensory neuropeptide in the nasal mucosa from patients with nonallergic chronic rhinitis. In: The New frontiers of otorhinolaryngology in europe; 1992; Sorrento, Italy: Monduzzi Editore S.p.A., Italy; 1992. p. 59-63.
77. Wolf G, Anderhuber W, Hauser-Kronberger C, Saria A. [Treatment of nonspecific hyper-reflectory rhinopathy (vasomotor rhinitis) with capsaicin]. Laryngorhinootologie 1995;74(5):289-93.
78. Riechelmann H, Davris S, Bader D. [Treatment of perennial non-allergic rhinopathy with capsaicin]. Hno 1993;41(10):475-9.
79. Marabini S, Ciabatti PG, Polli G, Fusco BM, Geppetti P. Beneficial effects of intranasal applications of capsaicin in patients with vasomotor rhinitis. Eur Arch Otorhinolaryngol 1991;248(4):191-4.
80. Ruffoli R, Fattori B, Giambelluca MA, Soldani P, Giannessi F. Ultracytochemical localization of the NADPH-d activity in the human nasal respiratory mucosa in vasomotor rhinitis. Laryngoscope 2000;110:1361-1365.
81. Broms P, Malm L. Oral vasoconstrictors in perennial non-allergic rhinitis. Allergy 1982;37(2):67-74.
82. Lieberman P. Treatment update: nonallergic rhinitis. Allergy Asthma Proc. 2001;22(4):199-202.
83. Wight RG, Jones AS, Beckingham E, Andersson B, Ek L. A double-blind comparison of intranasal budesonide 400 micrograms and 800 micrograms in perennial rhinitis. Clin Otolaryngol 1992;17(4):354-8.
84. Scadding GK, Lund VJ, Jacques LA, Richards DH. A placebo-controlled study of fluticasone propionate aqueous nasal spray and beclomethasone dipropionate in perennial rhinitis: efficacy in allergic and non-allergic perennial rhinitis. Clin

Exp Allergy 1995;25(8):737-43.
85. Banov CH, Lieberman P. Efficacy of azelastine nasal spray in the treatment of vasomotor (perennial nonallergic) rhinitis. Ann Allergy Asthma Immunol 2001;86(1):28-35.
86. Gehanno P, Deschamps E, Garay E, Baehre M, Garay RP. Vasomotor rhinitis: clinical efficacy of azelastine nasal spray in comparison with placebo. ORL J Otorhinolaryngol Relat Spec 2001;63(2):76-81.
87. Ciprandi G. Treatment of nonallergic perennial rhinitis. Allergy 2004;59 (Suppl. 76):16-23.
88. Tan R, Corren J. Optimum treatment of rhinitis in the elderly. Drugs Aging 1995;7(3):168-175.
89. Jones AS, Lancer JM, Moir AA, Stevens JC. The effect of submucosal diathermy to the inferior turbinates on nasal resistance to airflow in allergic and vasomotor rhinitis. Clin Otolaryngol 1985;10(5):249-52.
90. McCombe AW, Cook J, Jones AS. A comparison of laser cautery and sub-mucosal diathermy for rhinitis. Clin Otolaryngol 1992;17(4):297-9.
91. Moore JR, Bicknell PG. A comparison of cryosurgery and submucous diathermy in vasomotor rhinitis. J Laryngol Otol 1980;94(12):1411-3.
92. al-Samarrae SM. Treatment of 'vasomotor rhinitis' by the local application of silver nitrate. J Laryngol Otol 1991;105(4):285-7.
93. Ibragimov GT, Rasunova AK. [Treatment of patients with vasomotor rhinitis using snake venom]. [RUSSIAN]. Vestn Otorinolaringol 1987;1:39-41.
94. Golding-Wood PH. Vidian neurectomy and other trans-antral surgery—1970. Laryngoscope 1970;80(8):1179-89.
95. Golding-Wood PH. Vidian neurectomy: its results and complications. Laryngoscope 1973;83(10):1673-83.
96. Grote JJ. The autonomic innervation of the nasal mucosa . Nijmegen: University of Nijmegen; 1974.
97. Krajina Z. Critical review of Vidian neurectomy. [Review]. Rhinology 1989;27(4):271-6.
98. Krant JN, Wildervanck de Blecourt P, Dieges PH, de Heer LJ. Long-term Results of Vidian Neurectomy. Rhinology 1979;17(4):231-5.
99. Lekes MD. Rhinitis during pregnancy and rhinitis medicamentosa. Otolaryngol Head Neck Surg. 1992: 107(6 Pt 2):845-848

Chapter II

Aim of the study

The introduction to this thesis summarizes the literature for known causes of nonallergic noninfectious rhinitis and possible treatments. Also possible pathophysiological mechanisms of idiopathic rhinitis are discussed.
The fact that still about 50% of the patients with a nonallergic noninfectious rhinitis have to be classified as suffering from idiopathic rhinitis, or rather e causa ignota, is a continuing and frustrating burden for almost every clinicaly active ENT specialist and allergologist. This, combined with the limited and frequently insufficient treatment options currently available, was the guideline for the research described in this thesis.

The research questions addressed in this thesis are:

1 Is local, intranasal capsaicin a safe and effective therapy in idiopathic rhinitis?
2 Is it possible to develop an effective capsaicin treatment regimen that is more patient and physician friendly compared with those known from literature?
3 Is idiopathic rhinitis a chronic inflammatory disorder?
4 Do inflammatory cells and or sensory neurons play a role in the pathophysiology of idiopathic rhinitis?
5 What is the direct mode of action of local capsaicin on nasal mucosa cell counts and neurogenic staining.

The first two questions are addressed in chapter III and IV by two double-blind placebo controlled trials evaluating the treatment effect of intranasal capsaicin against placebo for idiopathic rhinitis patients. In chapter IV specific attention is paid to safety data and olfactory function during and after intranasal capsaicin treatment.

The third and fourth question are addressed in chapter V and VI. In chapter V mucosal inflammatory cell densities in nasal biopsies of idiopathic rhinitis patients are compared with nasal biopsies from healthy controls. The

involvement of inflammatory effector cells, the possibility of local allergy and possible neurogenically induced mast cell degranulation are studied. In chapter 5 the long term effects of intranasal capsaicin on the cellular homeostasis and overall neurogenic staining of the nasal mucosa are studied in a double-blind placebo controlled fashion.

The last question is addressed to in chapter VII with a double-blind placebo controlled biopsy study comparing the effect of intranasal capsaicin against placebo on nasal mucosa cellular homeostasis and neurogenic staining 15 minutes and 1 hour after provocation.

CHAPTER III

Intranasal capsaicin is efficacious in nonallergic, noninfectious perennial rhinitis. A placebo-controlled study.

H.M. Blom, J.B. van Rijswijk, I.M. Garrelds, P.G.H. Mulder, T. Timmermans and R. Gerth van Wijk.

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ABSTRACT

Background: Several authors described capsaicin, the pungent substance in red pepper, as an efficacious therapy for idiopathic rhinitis (IR). Repeated capsaicin application induces peptide depletion and specific degeneration of the unmyelinated sensory C-fibres in the nasal mucosa.
Methods: We performed a placebo-controlled (NaCl 0.9%) study with 25 IR patients. Daily record charts and visual analogue scales (VAS) were used for clinical evaluation. Nasal lavages were obtained before, during, and after treatment.
Results: There was a significant and long-term reduction in the VAS scores in the capsaicin group. No significant difference was found between the placebo and capsaicin treated groups for the mean group concentrations of leukotriene (LT) C4/D4/E4, prostaglandin D2 (PGD2), and tryptase. The levels of mast cell mediators, tryptase and PGD2, and leukotrienes, mediators derived from a variety of inflammatory cells, were low at baseline and comparable with levels observed in nasal lavages obtained from normals.
Conclusion: As involvement of inflammation could not be demonstrated, it is not surprising that capsaicin has no effect on inflammatory mediators. This suggests that inflammatory cells do not play a major part in the pathogenesis of IR.

INTRODUCTION

The knowledge of idiopathic rhinitis (IR) or vasomotor rhinitis is limited. This condition is unrelated to allergy, infection, structural lesions and / or other systemic diseases (1). The diagnosis is made by exclusion. Patients within this classification may complain of symptoms such as sneezing, watery rhinorrhoea and/or nasal obstruction. Treatment of this condition is more difficult than that of allergic rhinitis, a disease which can be relieved by use of antihistamines and nasal steroids.

The pathophysiology of non-allergic rhinitis is largely unknown (1). Several hypotheses have been put forward. A subgroup of patients may react to cold dry air with release of inflammatory mediators from mast cells involving a non-IgE-dependent mechanism (2). Inflammatory cells appears to play a minor part in the vast majority of patients (3). However, Knani reported a significant increase in tryptase levels, and increased levels of LTC4 and PGD2 in nasal lavage in symptomatic IR patients vs control subjects (4). Neurogenic mechanisms may be important since some patients, who react with watery discharge to spices and change of temperature, may benefit from use of anticholinergics (5).

Lacroix has shown that repetitive administration of capsaicin - the pungent agent in hot pepper - reduces nasal symptoms in patients with a rhinosinusitis, for which they underwent sinus surgery, or patients suffering from a drug-induced rhinitis (6). This reduction is accompanied by a decrease in positive immunoreactivity to calcitonin gene-related peptide (CGRP) in nasal biopsies. This observation is consistent with the observation that capsaicin induces peptide depletion and specific degeneration of the sensory C-fibres in the nasal mucosa of rodents (7). Several studies have been published showing that capsaicin desensitization might be an important therapeutic modality in IR (6,8-10). However, no placebo-controlled studies have been performed. Moreover, the reported studies lack well-defined criteria for having IR, with the risk of heterogeneity of the patients used.

The purpose of this study was to evaluate capsaicin treatment in a placebo-controlled fashion using a homogeneous group of well-characterized patients suffering from IR. Second, by measuring mediators of inflammation in nasal lavage fluid, we investigated the involvement of inflammation in IR and the

possible modulation by capsaicin.

METHODS

Subjects

Patients were admitted to the study if they had a history of nasal complaints such as nasal obstruction, sneezing, and rhinorrhoea for a period of over 1 year which could not be attributed to allergic rhinitis, nasal or paranasal sinus infection, anatomical disorders affecting nasal function, pregnancy or lactation and / or systemic disorders (Table 1). They were non-smokers not using medication affecting nasal function. Patients with nasal polyps were excluded, since they may belong to a different pathophysiological group and their polyps may contribute to a higher symptom score for nasal blockage and/or rhinorrhoea. Thirty-five patients, with the diagnosis of IR, scored their nasal complaints for a period of 2 weeks using a daily record chart (DRC) (Table 2) (11). In affected patients periods of either nasal discharge, and/or sneezing and/or congestion had to persist for an average of at least 1 h per

Table 1.

Inclusion criteria

- Age between 16 and 64 years.
- Negative skin prick test: house dust mite, tree pollen mix, grass pollen mix, bijvoet, alternaria, aspergillus, cladosporium, penicillum, dog, cat, parakeet, rabbit, hamster, horse, guinea pig. (ALK-Diephuis, Holland)
- Negative Phadiatop (Pharmacia, Uppsala, Sweden)
- Symptoms for more than 1 year.
- Periods of nasal discharge, sneezing and congestion for an average of at least 1 h per day for at least 5 days during a period of 14 days.

Exclusion criteria

- The use of systemic or inhaled corticosteroids within the previous month.
- Use of inhaled sodium cromoglycate or nedocromil sodium within the previous month.
- Use of astemizole within the previous month.
- Inability of the patient to stop taking medication affecting nasal function.
- A serious and/or unstable disease.
- Nasal surgery within the previous 6 weeks.
- Nasal polyps or a history of nasal polyps.
- Significant anatomical abnormalities affecting nasal function.
- Nasal or paranasal sinus infection (abnormal sinus X-ray).
- Pregnancy or lactation
- Abnormal findings at physical examination
- Abnormal laboratory results for:

blood: Na, K, Ca, total protein, albumin, urea, creatinine, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gammaglutamyl transpeptidase, hemoglobin, red blood cell count, plasma cell volume, mean corpuscular volume, platelets, total white blood cellcount, neutrophils, lymphocytes, monocytes, eosinophils, basophils.
urine: blood, protein, glucose.

Table 2.

Possible scores on the daily record chart	
Nasal blockage: (not being able to breathe freely through the nose) Clear nasal discharge: (runny nose)	0 = absent 1 = between 0-1h per half day 2 = between 1-2 h per half day 3 = more than 2 h per half day
Sneezing Coughing	0 = absent 1 = less than 5 periods per half day 2 = between 5-10 periods per half day 3 = more than 10 periods per half day
Mucus production: (yellow, green or brown)	0 = absent 1 = present

day for at least 5 days during a period of 14 days. Coughing and coloured mucus production were used as indicators of upper airway infection and thus used as exclusion criterion. The duration of complaints during the day was used as the prime criterion for further study. Twenty-five of the 35 patients were found eligible for our study and participated under conditions of informed consent (male/female: 16/9); mean age was 36 years (18-60). Procedures were approved by the local Medical Ethics Committee.

Study design

Patients were randomized and treated with placebo (11 persons) or capsaicin (14 persons) as depicted in Fig. 1a. This study was performed in a double-blind placebo-controlled fashion. Three applications of xylometazolinehydrochloride 0.1% (Otrivin® (1 mg/mL Zyma, Breda, Holland), nebulizator) were given for decongestion in each nostril. The nasal airway was anesthetized by three applications (10 mg/puff) of lidocainebase (100 mg/mL) (Xylocaine® 10% spray (Astra, Rijswijk, Holland)) in each nostril. To ensure good anaesthesia a pause of 15 min was introduced. Lips, columella, and philtrum were covered with a petrolatum/lanolin/glycerin salve. Capsaicin test puff was done in an exhaust hood to avoid eye irritation. Patients were instructed to inhale deeply before, hold their breath during and to exhale after substance application. The capsaicin solution (0.1 mmol/L)

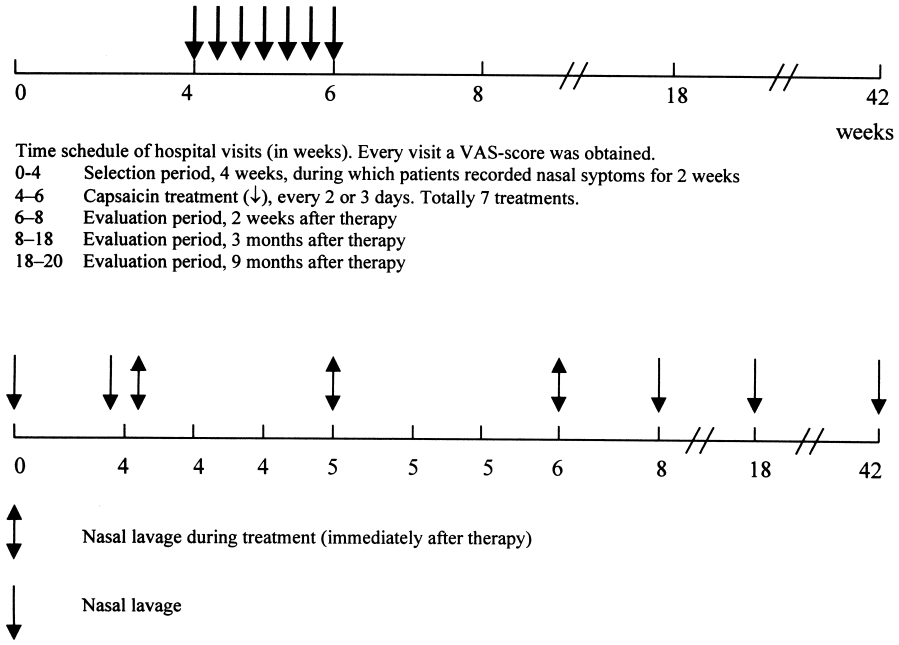

Figure 1.

consisted of pelargonic acid vanillylamide (Fluka, Buchs, Germany) dissolved in 3 mL alcohol (96%) and diluted in 1 L NaCl solution (0.9%) (Wolf, pers. comm.). For 'placebo therapy' we used NaCl solution (0.9%). During provocation 0.5 mL solution was sprayed in each nostril (0.15 mg capsaicin). Blood and urine samples were taken during visits 1 and 9 (Table 1) to monitor changes during therapy. At every visit the subjects rated overall nasal symptoms since the last visit on a visual analogue scale (VAS) (0-10 cm, 0 represented absence of symptoms and 10 represented high intensity of symptoms). DRC scoring was continued until 2 weeks after treatment. Nasal lavage was performed according to the method of Greiff (12), using a modified nasal pool device. Experience with nasal lavage has been obtained in several studies (13). Nasal lavage was performed (Fig. 1b) with 14 mL saline, preheated to 37 °C. Seven millilitres of saline were instilled into each nostril. After 10 s, the lavage fluid was expelled and collected in tubes, stored on ice and centrifuged for 10 min at 400 ×g. The supernatant was stored at -20 °C until analysis.

Mediator assays

The levels of leukotriene (LT) C4/D4/E4 and prostaglandin (PG) D2 were measured by Biotrak® and Radioimmunoassay (RIA), respectively (Amersham, UK). The limits of sensitivity of the assays were (almost equal to) 10 pg/mL for both assays. Cross reactivity of LTC4/D4/E4 assay: LTC4 (100%), LTD4 (100%), LTE4 (70%), LTB4 (0.4%) and prostaglandins (< 0.006%); PGD2 assay: PGD2 (100%), PGJ2 (7%), TxB2 (0.3%), PGF2(alpha) (0.04%) and other prostaglandins (< 0.02%).

Tryptase was determined by RIA according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). The detection limit was 0.5 mU/mL. Crossreactivity for heparin (< 0.01%) (14).

Statistical analysis

VAS data during treatment were analysed using a repeated measures analysis of variance. In the model, time was included as a quantitative variable; the interaction between time and treatment group was also included. Hence, a difference in time trend between the two treatment groups can be estimated and tested. The within-subject (co)variance matrix of the residuals is supposed to be unstructured. Leukotrienes, prostaglandin D2, and tryptase are analysed after log transformation.

Measurements after treatment (visits 9, 10, and 11) were analysed separately as changes from baseline using t-tests, between groups (unpaired) as well as within groups (paired). DRC data are summarized as within patient averages over 2-week periods: a first period before randomization/treatment, a second period after randomization (during therapy) and a third period after cessation of treatment. Between groups differences are tested using the Mann-Whitney U-test. P-values < 0.05 were considered significant.

RESULTS

The application of Xylocaine® spray in the nasal airway was immediately followed by a painful sensation that was described by all subjects as most unpleasant. Patients did not complain of irritation of nose and lips during or after capsaicin/placebo application.

One of the 14 capsaicin patients could not continue after three capsaicin applications because of influenza with fever.

Symptom scores

DRC

The mean score (± standard error of the mean) on the DRC of the included patients was 2.0 (± 0.049) for blockage, 1.4 (± 0.044) for clear nasal discharge, and 1.5 (± 0.033) for sneezing before therapy. No significant difference was found for the individual symptoms as well as the mean sumscore before, during or after therapy.

VAS

The mean of VAS is shown in Fig. 2. There was no significant difference between the groups in the VAS score before treatment. During treatment a smaller trend with time (-0.40 per two days) was seen in the capsaicin group than in the placebo group (+ 0.019 per two days), the difference being significant (P = 0.0007). At visits 9, 10 and 11 the difference between the groups remained significant. Also, the difference from baseline remained

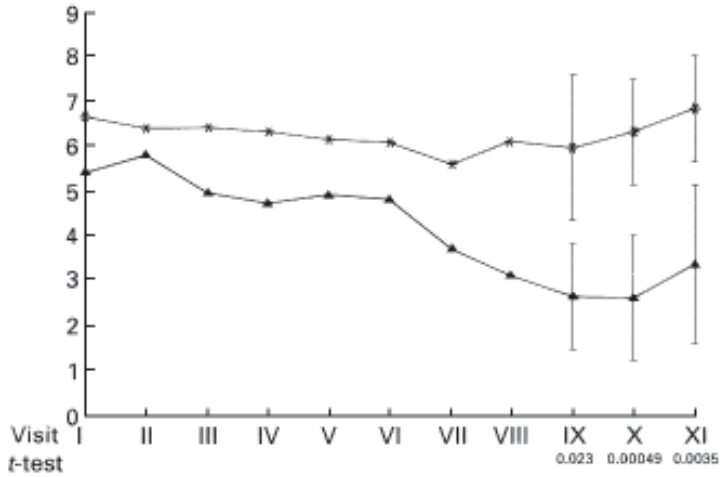

Fig. 2. The mean of the symptom score measured on a V.A.S. (0-10 cm, 0 represented absence of symptoms and 10 severe intensity of symptoms) for nasal complaints. Y error bars indicate 2.07 SE. black up pointing small triangle: Capsaicin. star (upper curve): Placebo.

significant within the capsaicin group from visit 9 onwards. This was not the case in the placebo group (Fig. 2).

Nasal lavage

The median return, of the 14 mL NaCl instilled, was 10 mL. The mean baseline levels (± standard error of the mean) of tryptase, LTC4/D4/E4, and PGD2 were 1.98 (± 0.422) mU/mL, 7.70 (± 3.10) pg/mL, and 16.2 (± 2.00) pg/mL, respectively, in the nasal lavage fluid of the treated group.
The mean baseline levels (± standard deviation) of tryptase, LTC4/D4/E4, and PGD2 were 2.09 (± 0.611) mU/mL, 2.62 (± 1.12) pg/mL, and 16.4 (± 2.85) pg/mL, respectively, in the nasal lavage fluid of the placebo group.
During treatment no significant difference in time trend between the two groups was found for the concentrations of tryptase, LTC4/D4/E4, and prostaglandin D2 in the nasal lavage fluid. At visits 9, 10 and 11 no significant changes from baseline or significant differences between the groups were found neither.

Safety data

None of the patients had a relevant change of blood and/or urine chemistry outside the normal range.

DISCUSSION

There is a dearth of information regarding the pathophysiology of IR. The limited understanding of this condition hampers the development of therapeutic modalities. An imbalance in the non-adrenergic, non-cholinergic peptidergic neuronal system has been proposed as the underlying mechanism of IR (15). Treatment with capsaicin may fit in with this hypothesis (16). This study showed that seven treatments in a 14-day period ameliorated symptoms during a follow-up of 9 months. It is possible that reduction of symptoms will last longer; however, we feel that it is unethical to maintain a placebo-treatment for many months, so we ended the trial after 9 months of follow-up. This long-term placebo-controlled study confirmed the efficacy and safety observations made during open uncontrolled studies (6,8-10).

The study has several limitations. The study was designed in a placebo-controlled double-blind fashion. However, we did not expect that we could blind the treatment for the patients, as this was considered impossible by several authors. In contrast to our expectations patients complained severely about the Xylocaine^{R} spray. Therefore, they were not able to discriminate between the active and the placebo substance. Furthermore the immediate respons to treatment did not permit us to discriminate between patients receiving capsaicin or placebo.
Second, since we used saline as placebo treatment rather than the solution used for dissolving capsaicin (which contained saline with 0.3% alcohol 96%), we cannot exclude the possibility that an effect of alcohol biased the therapeutic efficacy of capsaicin. It is, however, unlikely that instillation of these minute quantities of alcohol will induce a significant reduction in nasal symptoms during 9 months. Moreover, saline containing a fivefold dose of 1.5% alcohol has no effect on nasal conductance (17).
Finally we encountered a discrepancy between the reduction in VAS score and the absence of effect on DRC, which might be explained by the difference in nature between the scoring methods. The VAS scores the severity of the complaints whilst the DRC scores the duration of the complaints.
Furthermore, as the study proceeded patients compliance (in filling in the DRC) seemed to grow less, since scoring the DRC is a time-consuming and daily returning task. At the end of the trial some patients even reported that they had filled in their DRCs all at once just prior to their hospital visit.
In contrast, the VAS score is a quick and easy method for the patient. Also the fact that the placebo group showed no evidence of improvement in the VAS combined with the finding that the duration of the treatment's effect is quite impressive and consistent with what all the previous uncontrolled studies have suggested (6,8-10), we feel the VAS is more reliable than the DRC.
In animals capsaicin stimulates sensory C-fibres with the resultant release of substance P (SP) (7,18) and calcitonin gene related peptide (CGRP) (19). However, after several stimulations this is followed by depletion of these fibres and results in desensitization to capsaicin and other stimuli (16). As tachykinins (20) and capsaicin (21) induce the recruitment of inflammatory cells in the nose in allergic rhinitis and SP releases histamine and TNF(alpha) from peritoneal mast cells in animals (22), capsaicin may modulate inflam-

mation of the nasal mucosa. The levels of tryptase, PGD2, and leukotrienes, mediators derived from several inflammatory cells such as eosinophils, basophils, and mast cells (23,24), were low at baseline and comparable with levels observed in nasal lavages obtained from normals (C. de Graaf-in 't Veld, pers. comm.). This contrasts with the results presented by Knani (4) However, six out of 14 patients in Knani's study showed a prominent eosinophilia in nasal secretions and may have been NARES patients. Our data concord with the findings of Roche, however this paper describes the results in asymptomatic patients. As involvement of inflammation could not be demonstrated, it is not surprising that capsaicin has no effect on inflammatory mediators. Perhaps the absence of inflammation, also demonstrated in a recent study (3), is an explanation of the moderate efficacy of nasal steroids in non-allergic rhinitis.

To conclude, capsaicin is an efficacious substance in the treatment of IR. In our placebo-controlled study a therapeutical effect lasted more than 9 months. No effect was found on inflammatory mediators. No adverse side-effects were noted.

REFERENCES

1. Philip G, Togias AG. Nonallergic rhinitis. Pathophysiology and models for study. [Review]. Eur Arch Otorhinolaryngol Suppl 1995;1:S27-32.
2. Togias A, Naclerio RM, Proud D et al. Studies on the allergic and nonallergic nasal inflammation. [Review]. J Allergy Clin Immunol 1988; 81(5 Pt 1):782-90.
3. Blom HM, Godthelp T, Fokkens WJ et al. Mast cells, eosinophils and IgE-positive cells in the nasal mucosa of patients with vasomotor rhinitis. An immunohistochemical study. Eur Arch Otorhinolaryngol Suppl 1995; 1:S33-9.
4. Knani J, Campbell A, Enander I et al. Indirect evidence of nasal inflammation assessed by titration of inflammatory mediators and enumeration of cells in nasal secretions of patients with chronic rhinitis. J Allergy Clin Immunol 1992; 90(6 Pt 1):880-9.
5. Dolovich J, Kennedy L, Vickerson F, Kazim F. Control of the hypersecretion of vasomotor rhinitis by topical ipratropium bromide. J Allergy Clin Immunol 1987; 80(3 Pt 1):274-8.
6. Lacroix JS, Buvelot JM, Polla BS, Lundberg JM. Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy

1991; 21:595-600.
7. Lundblad L, Hua XY, Lundberg JM. Mechanisms for reflexive hypertension induced by local application of capsaicin and nicotine to the nasal mucosa. Acta Physiol Scand 1984; 121:277-82.
8. Wolf G, Anderhuber W, Hauser-Kronberger C, Saria A. [Treatment of nonspecific hyper-reflectory rhinopathy (vasomotor rhinitis) with capsaicin]. [German]. Laryngorhinootologie 1995; 74:289-93.
9. Riechelmann H, Davris S, Bader D. [Treatment of perennial non-allergic rhinopathy with capsaicin]. [German]. HNO 1993; 41:475-9.
10. Marabini S, Ciabatti PG, Polli G, Fusco BM, Geppetti P. Beneficial effects of intranasal applications of capsaicin in patients with vasomotor rhinitis. Eur Arch Otorhinolaryngol 1991; 248:191-4.
11. Norman PS, Winkenwerder WL, Lichtenstein LM. Trials of alum-precipitated pollen extracts in the treatment of hay fever. J Allergy Clin Immunol 1972; 50:31-44.
12. Greiff L, Pipkorn U, Alkner U, Persson CG. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. Clin Exp Allergy 1990; 20:253-9.
13. Gerth van Wijk R. Nasal hyperreactivity. Thesis. Erasmus University, Rotterdam, The Netherlands. 1991.
14. Garrelds IM, de Graaf-in 't Veld C, Jansen APH, Gerth van Wijk R, Zijlstra FJ. Effect of fluticasone prpionate aqueous nasal spray treatment on platelet activating factor and eicosanoid production by nasal mucosa in patients with a house dust mite allergy. Med Inflammation 1994; 3:381-5.
15. Albegger K. [Current aspects of nasal hyperreactivity]. [Review] [German]. HNO 1988; 36:389-98.
16. Wolf G. [New aspects in the pathogenesis and therapy of hyperreflexive rhinopathy]. [Review] [German]. Laryngol Rhinol Otol 1988; 67:438-45.
17. Roche N, Lurie A, Authier S, Dusser DJ. Nasal response to capsaicin in patients with allergic rhinitis and in healthy volunteers: effect of colchicine. Am J Respir Crit Care Med 1995; 151:1151-8.
18. Lundblad L, Saria A, Lundberg JM, Anggard A. Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons. Acta Otolaryngol 1983; 96(5-6):479-84.
19. Stjarne P, Lacroix JS, Anggard A, Lundberg JM. Release of calcitonin gene-related peptide in the pig nasal mucosa by antidromic nerve stimulation and capsaicin. Regul Pept 1991; 33:251-62.
20. Braunstein G, Fajac I, Lacronique J, Frossard N. Clinical and inflammatory responses to exogenous tachykinins in allergic rhinitis [published erratum appears

in Am Rev Respir Dis 1993 Dec;148(6 Pt 1):following 1700]. Am Rev Respir Dis 1991; 144(3 Pt 1):630-5.
21. Philip G, Sanico AM, Togias A. Inflammatory cellular influx follows capsaicin nasal challenge. Am J Respir Crit Care Med 1996; 153:1222-9.
22. Ansel JC, Brown JR, Payan DG, Brown MA. Substance P selectively activates TNF-alpha gene expression in murine mast cells. J Immunol 1993; 150:4478-85.
23. Owen W Jr., Soberman RJ, Yoshimoto T et al. Synthesis and release of leukotriene C4 by human eosinophils. J Immunol 1987; 138:532-8.
24. Schleimer RP, MacGlashan D Jr., Peters SP et al. Characterization of inflammatory mediator release from purified human lung mast cells. Am Rev Respir Dis 1986; 133:614-7.

CHAPTER IV

Intranasal capsaicin reduces nasal hyperreactivity in idiopathic rhinitis: a double-blind randomized application regimen study.

J.B. van Rijswijk, E.L. Boeke, J.M. Keizer, P.G.H. Mulder, H.M. Blom, and W.J. Fokkens

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ABSTRACT

Background: In a recent study, we showed that intranasal capsaicin spray gives a significant and long-term reduction of symptoms in nonallergic non-infectious perennial rhinitis patients. However, in daily practice, the studied application regimen proved to be impractical because of the large number of visits required in a short period of time. In the present study, we conducted a double-blind double-dummy parallel groups trial to determine whether a more practical capsaicin application schedule is equally effective.
Methods: Thirty patients were randomized into two different treatment regimens: one group received capsaicin five times the first day at one-hour intervals. This was followed by a placebo dummy once every second or third day for a total of five treatments 2 weeks after the capsaicin application (group A). The other group (B) received the placebo dummy five times on the first day followed by capsaicin once every second or third day for a total of five treatments 2 weeks after the placebo application.
Results: The visual analogue scale scores for overall nasal symptoms, rhinorrhea and nasal blockage showed significant decrease after the start of treatment in both groups, with a significantly steeper decrease in group A. A significant reduction in cold dry air dose responsiveness was also found up to 9 months after therapy in both groups, reflecting a decrease in nasal hyperreactivity. No significant changes in safety data (smell, blood pressure, heart rate) were found.
Conclusions: We conclude that intranasal capsaicin seems safe to use and that five treatments of capsaicin on a single day is at least as effective as five treatments of capsaicin in 2 weeks.

INTRODUCTION

Perennial rhinitis is a common disorder causing significant morbidity. Chronic rhinitis can be due to common factors such as mechanical obstruction, allergy or less common factors such as xylometazoline abuse or cystic fibrosis. But there are several types of chronic rhinitis of which the pathophysiology is not yet fully elucidated. Syndromes of chronic rhinitis with an unknown aetiology include nonallergic noninfectious perennial rhinitis, formerly referred to by us as NANIPER. In accordance with the ''World Health Organisation Initiative, Allergic Rhinitis and its Impact on Asthma'' (1), henceforth we will be using the term idiopathic rhinitis (IR) to describe this pathology. IR, formerly also called vasomotor rhinitis, is a diagnosis of exclusion and is given to patients who suffer from perennial nasal congestion, rhinorrhea and/or sneezing with no identifiable aetiology. IR is unrelated to allergy, infection, structural lesions, polyposis and other systemic diseases (2). Patients with nonallergic rhinitis with eosinophilia syndrome (NARES) form another subgroup of IR patients. They have significant mucosal eosinophilia (a nasal smear with more than 25% eosinophils (3)) and respond well to nasal corticoster oids (4). In previous studies, we found hardly any patients with NARES in our IR patient groups, probably because we only selected IR patients in whom no therapeutic effect had been achieved with nasal corticosteroids (5, 6).

The population incidence of IR is estimated at 2-4 %(1). The impact on the quality of life of patients suffering from chronic rhinitis is significant, a fact that is often underestimated and neglected (7). In many patients, treatment with anti-histamines, nasal steroids or even nasal surgery is not beneficial (2).

The pathophysiology of IR is largely unknown. Several hypotheses have been put forward. Inflammatory cells appear to play a minor part in the vast majority of patients (5, 6). It is assumed that neurogenic mechanisms play an important role (8). Neuropeptides (CGRP, SP, etc.) are released from peptidergic neurons in the nasal mucosa after activation by unspecific stimuli, and can be responsible for the symptoms of IR(9-11). Several studies have been published showing a therapeutic effect in IR patients for repeated topical applications of capsaicin (12-15). Capsaicin, the pungent agent in hot

pepper, is known for its degeneration/desensitization effect on peptidergic sensory C-fibres, possibly explaining its therapeutic effect (16, 17).
In a recent paper, we showed that repeated administration of capsaicin in a double blind placebo-controlled trial led to a significant and long-term reduction of symptoms (15). That study showed that intranasal capsaicin application once every second or third day for a total of 7 days has a significant, long lasting beneficial effect compared with placebo. However, in daily practice, the studied application regimen proves to be unpractical for both patient and physician because of the large number of visits required in a short period of time.
The purpose of the present study was to conduct a double-blind double-dummy parallel groups trial to determine whether a more practical capsaicin application schedule is equally effective. Furthermore, we collected more safety data (blood pressure, heart rate) and paid specific attention to nasal capsaicin sensitivity, mucosal sensibility and olfactory function before and after therapy.

MATERIALS AND METHODS

Subjects
Patients were admitted to the study if they had a history of nasal complaints such as nasal obstruction, sneezing and/or rhinorrhea for a period of over 1 year, which could not be attributed to allergic, nasal or paranasal infection, anatomical disorders affecting nasal function, pregnancy or lactation and/or systemic disorders (Table 1). They had to have used a nasal corticosteroid spray for at least 6 weeks without any beneficial effect on their nasal symptoms. They were non-smokers not using medication affecting nasal function. All patients underwent nasendoscopy and patients with nasal polyps were excluded.
Patients with a diagnosis of IR scored their nasal complaints for a period of 2 weeks using a daily record chart (DRC) (Table 2). They were included in the study if periods of either clear nasal discharge, and/or sneezing and/or congestion persisted for an average of at least 1 h a day for at least 5 days during a period of 14 days (18). Thirty patients participated under conditions of informed consent (male/female: 14/16); mean age was 36 years (16 – 65

Table 1. Inclusion and exclusion criteria.

Inclusion criteria
Age between 16 and 65 years.
Negative Phadiatop (Pharmacia, Uppsala, Sweden)
Symptoms for more than 1 year.
Periods of nasal discharge, sneezing and congestion for an average of at least 1 h per day for at least 5 days during a period of 14 days.
No beneficial effect of nasal corticosteroid spray (for a period of at least 6 weeks)
Exclusion criteria
Use of systemic or inhaled corticosteroids in the previous month.
Use of inhaled sodium cromoglycate or nedocromil sodium in the previous month.
Use of astemizole in the previous month.
Inability of the patient to stop taking medication affecting nasal function.
A serious and/or unstable disease.
Smoking (in the previous 6 months)
Nasal surgery in the previous 6 weeks.
Nasal polyps or a history of nasal polyps.
Significant anatomical abnormalities affecting nasal function.
Nasal or paranasal sinus infection (abnormal sinus X-ray).
Pregnancy or lactation

years). Procedures were approved by the local Medical Ethics Committee.

Study design

This study was performed in a double-blind randomized fashion. Patients were randomized 1 : 1 either for group A or for group B. For this purpose a computer generated randomization list was prepared in blocks of 8 randomly permuted allocations. On the basis of this list the double-blind medication was prepared by the local pharmacist. Patients in group A were first treated with capsaicin five times on a single day at 1-h intervals. After 2 weeks, they received a total of five treatments with dummy placebo once every second or third day. Patients of group B first received dummy placebo five times on a single day at 1-h intervals. This was followed 2 weeks later by a total of five treatments with capsaicin once every second or third day. The dummy placebos serve to ensure blindness of the study. The study design is shown in

Table 2. Design of the daily record chart for defining nasal symptoms in IR patients.

Possible scores on the daily record chart	
Nasal blockage:	0 = absent
(not being able to breathe freely through the	1 = between 0-1h per half day
nose)	2 = between 1-2 h per half day
Clear nasal discharge: (runny nose)	3 = more than 2 h per half day
Sneezing	0 = absent
Coughing	1 = less than 5 periods per half day
	2 = between 5-10 periods per half day
	3 = more than 10 periods per half day
Green/Yellow mucus production:	0 = absent
	1 = present

Fig. 1.
Each application of capsaicin or placebo was preceded by three applications of xylometzoline-hydrochloride 0.1% [Otrivin® nebulisator (1mg/ml Zyma, Breda, Holland)] in each nostril for decongestion. The nasal mucosa was then anaesthetized by three applications (10mg/puff) of lidocaine base [100mg/ml, Xylocaine® 10% spray (Astra, Rijswijk, Holland)] in each nostril. To ensure good anaesthesia a pause of 15 min was introduced. The lips, columella and philtrum were covered with a petrolatum/ lanolin/ glycerine salve. The capsaicin solution (0.1 mmol/l) consisted of 30.3 mg pelargonic acid vanillylamide dissolved in 3 ml alcohol (96%) and diluted in 1 l NaCl

Study-layout

Visit:	1	2	3	4	5	6	7	8	9	10	11	12
Day:	0	14	21	35				44	58	72	128	310
Therapy group A:			ccccc	p	p	p	p	p				
Therapy group B:			ppppp	c	c	c	c	c				
Nasal symp.VAS:	*	*	*	*	*	*	*	*	*	*	*	*
UPSIT:	*										*	
CDA:	*									*	*	*
Ac.Rhinometry:	*	*	*	*	*	*			*	*	*	*
PNIF:	*	*	*	*	*	*			*	*	*	*
Caps.sens.:		*		*				*		*	*	*
Mucosal sens.:	*										*	
BP/HR:	*		*			*		*			*	

Figure 1. Study-layout. c: Capsaicin application. At visit 3, five times at intervals of 1 h in group A; at visits 4 - 9, once every visit in group B. p: Placebo application. At visit 3, five times at intervals of 1 h in group B; at visits 4 - 9, once every visit in group A. BP blood pressure, HR heart rate.

solution (0.9%). As placebo, we used the capsaicin solvent only. During provocation, 0.27 ml of solution (three applications) was sprayed into each nostril with a metered nasal spray (0.09 ml per actuation, coefficient of variation 4%).
At every visit, the subjects rated the following four nasal symptoms during the last 3 days on four separate visual analogue scales (VAS) (0-10 cm, 0 cm represented an absence of symptoms and 10 cm represented highest intensity of symptoms): overall nasal symptoms, rhinorrhea, nasal obstruction and sneezing. DRC scoring was continued during administration with capsaicin and placebo until 4 weeks after the last treatment and 1 week before every follow-up visit thereafter.

Olfactory function
Olfactory function was measured before and after treatment using the University of Pennsylvania Smell Identification Test (UPSIT)(19).

Cold dry air provocation / nasal reactivity
Nasal reactivity (nasal patency, mucus production, and sneezing) was measured using standardized cold dry air provocation (CDA) before and after therapy. The dose steps for cold dry air were 12.5, 25, 50, 100, 200 and 400 l, comprising of a first step of 12.5 l/min and other steps of 25 l/min at, respectively, 1, 1, 2, 4, 8 and 16 minutes. The –10 ^{o}C air leaving the respiratory heat exchanger (Jaeger GmbH, Wurzburg, Germany) had a relative humidity of < 10 % and entered the nasal cavity by means of a specially designed nose cap (Respricare, The Hague, The Netherlands). As soon as a threshold dose resulting in 40% reduction of nasal patency and/or 0.5 g mucus production (cut-off lines) was reached, the provocation series was stopped (20).

Nasal patency: acoustic rhinometry and PNIF
Nasal patency was studied before, during and after therapy. The acoustic rhinometer Rhin2100 (RhinoMetrics, Denmark) was used to measure the first two minimal cross-sectional areas (MCA1 and MCA2) using contoured nose-adaptors. To reduce variability, three replicate measurements were done and the mean of these measurements (MMCA1 and MMCA2) was used for

further analysis.
Nasal patency was also studied on the basis of peak nasal inspiratory flow (PNIF) before, during and after therapy. On each occasion, three replicate PNIF measurements were done and the best one was used for further comparison/statistics.

Capsaicin sensitivity
Capsaicin sensitivity was measured before, during and after therapy by spraying capsaicin solution and placebo into both nostrils in a random order and asking the patient to point out which of the applications caused a pungent sensation (to discriminate the capsaicin solution from the placebo). Starting at 10^{-8} M, the capsaicin concentration was increased multiplicatively each time by the cubic root of 10**,** until the patient correctly distinguished the capsaicin solution from placebo three times in a row. This concentration indicated capsaicin sensitivity.

Mucosal sensibility
To study mucosal sensibility before and after therapy we touched the patients' nasal mucosa in a random order with a cotton wool stick (testing epicritic sensibility) and a thin metal rod (testing protopathic sensibility) and asked them to rate this sensation on a VAS (0-10 cm, 0 cm represented an absence of sensation and 10 cm represented highest intensity of sensation). This was done before and after therapy.

Blood pressure / Heart rate
During several visits before, during and after therapy, blood pressure (BP) and heart rate (HR) were measured in a sitting position by standard sphygmomanometry.

Statistical analysis
Total nasal symptom VAS score was the primary outcome variable. No formal power calculation underlies this sample size.
Nasal symptom VAS data (10 repeated measurements under treatment) were

analysed using repeated measures analysis of variance after log transformation with the baseline measurement as covariate. Exponential time trends, differences in time trends and, if no significant differences were found, constant differences in mean level between the two treatment groups were tested
The various DRC symptom scores are defined in Table 2. DRC symptom scores were aggregated in six periods by averaging the daily scores per period per patient. The following six periods were distinguished: a baseline period of 3 weeks (period 0), period 1 (week 4 and 5 after baseline), period 2 (week 7 and 8), period 3 (week 10), period 4 (week 17) and period 5 (week 41). These aggregated data were analysed using repeated measures analysis of variance with the baseline average as covariate, the period as a within-patient factor (with five levels) and treatment as a between-patient factor with two levels. The interaction between period and treatment was also tested. The residuals were assumed to have a Gaussian spatial covariance structure accounting for differences in time between the repeated measurements.
UPSIT data were analysed using analysis of covariance with the baseline measurement as covariate for between-group differences. Within-group changes from baseline were tested using the paired t-test.
CDA data were analysed after $\log_2$ transformation using repeated measures analysis of variance with the baseline measurement as covariate. By this transformation, effects are expressed in doubling dose units.
Mucosal sensibility VAS data and capsaicin sensitivity data were analysed using nonparametric tests: the Wilcoxon signed rank test for within group changes and the Mann-Whitney U-tests for differences between the two groups. For capsaicin sensitivity the paired Wilcoxon test was applied after log transformation.
Acoustic rhinometry, PNIF, heart rate, systolic and diastolic blood pressure were analysed using repeated measures analysis of variance with the baseline measurement as covariate. For acoustic rhinometry, the sum of right and left for MMCA1 (TMMCA1) and MMCA2 (TMMCA2) was taken. Linear time trends were tested, as well as differences in time trends between the two treatment groups.
For differences in heart rate, and in systolic and diastolic blood pressure between the two treatment groups, differences between the visits and the interaction between these factors were tested. The null hypothesis is that the

mean outcome variable does not change in time and is the same for both groups.
For all tests the significance level was set at 0.05. If appropriate, 95 % confidence intervals (CI) of between-groups differences in treatment effect are presented.

RESULTS

The application of Xylocaine^{R} 10% spray in the nasal airway was immediately followed by a painful sensation that was described by all subjects as most unpleasant.
Patients did not complain of irritation of nose and lips during or after capsaicin/placebo application. We feel this study was effectively blinded for both patients and investigator.
Pre-treatment baseline data for patient characteristics and for efficacy variables per group are shown in Table 3 for both groups.

Table 3. Baseline data

	Group A	Group B
Number of patients (male patients)	15 (6)	15 (8)
Age * (years)	33 (17 - 54)	37 (16 - 65)
VAS Overall nasal symptoms *	6.4 (2.5 - 9.5)	8.2 (2.8 - 9.7)
VAS Rhinorrhea *	4.3 (0.1 - 9.1)	5.2 (0.2 - 9.6)
VAS Obstruction *	6.7 (0.8 - 9.7)	7.2 (4.7 - 9.9)
VAS Sneezing *	1.8 (0.1 - 7.9)	3.5 (0.0 - 9.7)
VAS Epicritic sensibility *	2.5 (0.1 - 6.5)	4.6 (0.1 - 9.7)
VAS Protopathic sensibility *	1.9 (0.3 - 6.5)	4.1 (0.3 - 9.8)
TMMCA 1 # (cm^2)	1.3 (0.30)	1.0 (0.19)
TMMCA 2 # (cm^2)	1.3 (0.39)	1.0 (0.40)
PNIF # (l)	172 (66)	147 (71)
CDA threshold dose * (l)	50 (12.5 – 200)	25 (12.5 - 400)
Capsaicin sensitivity * (M)	$1.0\ 10^{-6}$ ($4.6\ 10^{-8}$ to $4.6\ 10^{-6}$)	$1.0\ 10^{-6}$ ($4.6\ 10^{-8}$ to $2.2\ 10^{-6}$)
# mean (standard deviation)	* median (range)	

Symptoms

Visual analogue scale. The improvement of the median for VAS 'overall nasal symptoms' for both groups is shown in Fig. 2. In both groups a significant improvement of overall nasal symptoms was observed (also described later). Note the improvement started within 2 weeks after start of the treatment with capsaicin (visit IV for group A and visit VIII / IX for group B).

In group A, the VAS score for 'overall nasal symptoms' decreased significantly by 3.2 % per week after the start of treatment and in group B by 1.3 %, the difference in time trend being significant (95 % CI: 0.4 - 3.5 % points; P = 0.016). The VAS score for 'rhinorrhea' decreased significantly after the start of treatment by 3.7 % per week in group A and by 2.6 % in group B, the difference in time trend not being significant (P = 0.26). However, there was a constant difference in VAS level: group B scored on average 2.1 times higher than group A (95 % CI: 1.4 - 3.2; P = 0.0014). The VAS score for 'obstruction' decreased significantly after the start of treatment by 3.2 % per week in group A and by 1.8 % in group B, the difference in time trend being significant (95 % CI: 0.01 - 2.9 % points; P = 0.0484). The VAS score for 'sneezing' decreased after the start of treatment by 2.8 % per week in group A and by 1.3 % in group B, the difference in time trend not being significant (95 % CI: -0.2 to 3.3 % points; P = 0.0916). Also no significant constant difference in VAS level was seen between the two groups (95 % CI for group B to A ratio: 0.8 - 3.6; P = 0.17). For all nasal symptoms, the within-group VAS score decrease was significant in groups A and B (all P-values smaller than 0.04).

Daily record chart. For rhinorrhea, a significant mean difference was found of 0.34 scale units (95 % CI: 0.01 - 0.67; P = 0.0424) in favour of group A. A significant time effect was also found (P = 0.0002), showing a decrease in DRC score from baseline. There was no evidence of a time by group interaction (P = 0.76).

No significant difference between the groups was found for nasal blockage (95 % CI: -0.38 to 0.30 scale units; P = 0.81). A significant time effect was found (P = 0.0095), showing a decrease in DRC score from baseline. There was no evidence of a time by group interaction (P = 0.24).

For all other DRC scores, no significant effects of time or treatment were found.

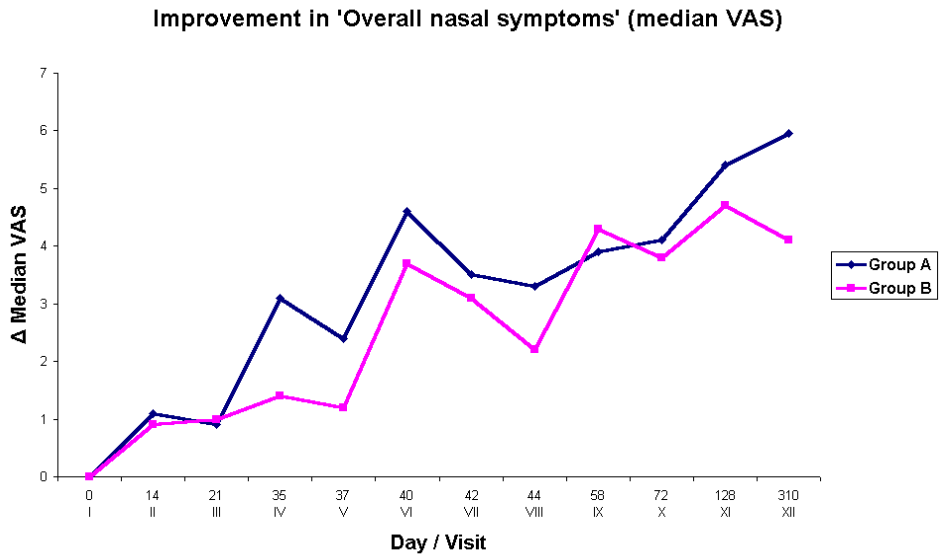

Figure 2. Improvement in median VAS for 'overall nasal symptoms'.
Patients in group A were first treated with capsaicin five times on a single day at intervals of 1 h (visit 3). After 2 weeks, they received a total of five treatments with placebo once every second or third day (visits 4-8). Patients of group B first received placebo five times on a single day at intervals of 1 h (visit 3). This was followed two weeks later by a total of five treatments with capsaicin once every second or third day (visits 4-8).
On every visit, they rated their 'overall nasal symptoms' on a visual analogue scale (0-10cm). The change in median VAS score from visit 1 is shown.
In both groups a significant improvement of overall nasal symptoms was observed. Note the improvement started within 2 weeks after start of the treatment with capsaicin (visit IV for group A and visit VIII / IX for group B).

Smell

UPSIT. The mean UPSIT score at visit 2 was 30 for group A (SD = 7.5) and 29 for group B (SD = 4.9). At visit 11, the mean score was 32 for group A (SD = 4.6) and 29 for group B (SD = 7.6). No significant changes were found in either group (P = 0.052 for group A and P = 0.67 for group B). Also between the two groups no significant difference (B – A) in level was found (95 % CI: -6.2 to 0.3; P = 0.082).

CDA Hyperreactivity

Cold dry air provocation. The median of the threshold dose for cold dry air provocation for both groups is shown in Fig. 3. In each group, there was a significant change from baseline (visit 1) at post-treatment visits 10, 11 and 12 (all P-values smaller than 0.0001). There was no significant treatment by visit interaction (P = 0.89). Also no significant constant difference in level between the two groups (B – A) was found (95 % CI: -1.6 to 0.3 doubling dose units; P = 0.20).

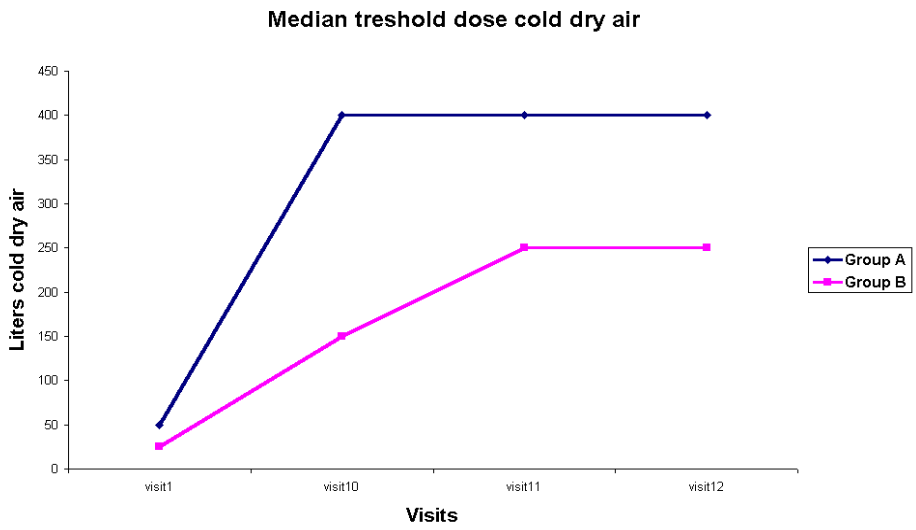

Figure 3. Median threshold dose for cold dry air.
Patients in group A were first treated with capsaicin five times on a single day at intervals of 1 h (visit 3). After 2 weeks they received a total of five treatments with placebo once every second or third day (visits 4-8). Patients from group B first received placebo five times on a single day at intervals of 1 h (visit 3). This was followed 2 weeks later by five treatments with capsaicin once every second or third day (visits 4-8).
Nasal reactivity was measured using standardized cold dry air provocation before (visit 1) and after (visit 10-12) therapy. The median threshold dose for cold dry air is shown.

Nasal patency

Acoustic rhinometry. The TMMCA1 increased significantly over time for group A and almost significantly for group B: by 0.014 cm^2 / week (P = 0.0027) for group A and by 0.009 cm^2 / week (P = 0.0596) for group B. The difference (B – A) in time trend was not significant (95 % CI: -0.018 to 0.007 cm^2 / week; P = 0.42). Also no significant constant difference in level between the two groups (B – A) was found (95 % CI: -0.06 to 0.07 cm^2; P = 0.86).

The TMMCA2 had no significant linear time trend in either treatment group (P = 0.78 for group A and P = 0.87 for group B). The difference in time trend (B – A) was not significant (95 % CI: -0.018 to 0.017 cm^2 / week; P = 0.94). Also no significant constant difference in level between the two groups (B – A) was found (95 % CI: -0.05 to 0.13 cm^2; P = 0.34).

Peak nasal inspiratory flow. The time trend decreased by 0.12 l/s per week in group A (P = 0.69) and increased by 0.30 l/s per week in group B (P = 0.30). The time trend in either treatment group was not significant, nor was the difference (B – A) in time trend between the two treatment groups (95 % CI: -0.39 to 1.21; P = 0.31). Also no significant constant difference in level between the two groups (B – A) was found (95 % CI: -65 to 3 l/s; P = 0.0732).

Sensitivity/sensibility nasal mucosa

Capsaicin sensitivity. No significant differences were found (in either group) between capsaicin sensitivity concentrations during and after therapy compared with the capsaicin sensitivity concentration before therapy (P > 0.42). In addition, no significant differences between the two groups were found for all visits (P > 0.09).

Mucosal sensibility. No significant changes from baseline were found in either treatment group for either epicritic (P = 0.44 for group A and P = 0.055 for group B) and protopathic sensibility (P = 0.57 for group A and P = 0.064 for group B). No significant difference was found between the two therapy groups (P = 0.51 for epicritic and P = 0.39 for protopathic sensibility).

Safety
Blood pressure / Heart rate. For heart rate, systolic and diastolic blood pressure, no significant effects of visit and group were found.

DISCUSSION

In a recent double blind placebo controlled study, we showed that repetitive capsaicin administration for a total of seven applications in 14 days gives a significant and long-term reduction of symptoms (15). The present study attempted to find a capsaicin application regimen that was more practical for both patient and doctor and at least equally effective as the previous one.
From our results, we can conclude that capsaicin treatment five times on a single day at intervals of 1 h (group A) is at least as effective as capsaicin treatment once every second or third day for a total of five treatments (group B). Some study parameters like the VAS scores for 'overall nasal symptoms', rhinorrhea and obstruction and the DRC score for rhinorrhea show even a significant better treatment effect for capsaicin treatment on a single day (group A).
A possible explanation for this is that, although the cumulative capsaicin dose was the same for both treatment groups, the concentration of capsaicin at the level of the nasal mucosa can reach much higher values for a longer period in the group that is treated five times in 1 day than in the group treated over a period of 5 days because of the wash-out effect in the latter group. This seems to be in agreement with the hypothesis that capsaicin leads to a selective degeneration/desensitization of peptidergic neurons in the nasal mucosa because higher concentrations of capsaicin for one longer time period can cause more degeneration/desensitization and reduce the opportunities for repair of these neurons than would be the case with five interrupted shorter periods. More effective degenerating/desensitising could mean that fewer neuropeptides will be released locally after irritating stimuli like cold dry air (antidromic effect). Also less sensory neural central stimulation might take place after irritating stimuli giving less central protective neural reflex mechanisms like secretion, extravasation and vasodilatation (orthodromic effect).
This provides an attractive explanation for the significant therapeutic cap-

saicin effect and the decrease in nasal hyperreactivity for cold dry air provocation.

Patients repeatedly treated with intranasal capsaicin solution are found to have reduced symptoms of pain and burning sensation with each successive capsaicin application as a sign of capsaicin desensitization (21). We had hoped to demonstrate, with our novel capsaicin sensitivity method, a decrease in capsaicin sensitivity after therapy as a result of the postulated capsaicin desensitization. However, this was not the case, perhaps because the instrument is not sensitive enough and misses small alterations. It is also possible that a learning effect in distinguishing between capsaicin and placebo masks a possible decrease in capsaicin sensibility. This latter phenomenon was observed in a group of normal individuals who were not treated with capsaicin but only repeatedly tested for capsaicin sensitivity (unpublished data).

Taking into account the results on the objective parameters of PNIF, acoustic rhinometry and CDA provocation in this study, it seems that the most important underlying pathophysiology that results in symptoms in IR patients is increased hyperreactivity of the nasal mucosa rather than decreased patency. This may also be an explanation for the correlation between the decrease in nasal reactivity measured by CDA provocation and nasal complaints, as well as for the absence of a significant change in PNIF and TMMCA2. Furthermore, it seems that the values of PNIF and acoustic rhinometry do not differ from values found in normal controls in other studies (22, 23).

During the trial, we paid a lot of attention to safety data in order to identify adverse side-effects. Because the concentration of capsaicin in the nasal mucosa could reach higher values in group A, a different, possibly more negative, effect on the safety data compared with group B was a possibility. We therefore collected values for blood pressure, heart rate, olfactory function and mucosal sensibility and compared them before and after treatment in and between the two groups. No significant differences were found so we conclude that local capsaicin application seems safe in both treatment regimens.

We conclude that local capsaicin nasal spray significantly reduces nasal complaints in IR patients and that five treatments of capsaicin on a single

day is at least as effective as five treatments of capsaicin in 2 weeks, and even more effective in the reduction of nasal complaints measured with VAS. We also conclude that intranasal capsaicin seems safe to use.

REFERENCES

1. Bousquet C. World Health Organisation Initiative Allergic Rhinitis and its Impact on Asthma (ARIA). Geneve: World Health Organisation; 2001.
2. Philip G, Togias AG. Nonallergic rhinitis. Pathophysiology and models for study. Eur Arch Otorhinolaryngol Suppl 1995;1(3):S27-32.
3. Mikaelian AJ. Vasomotor rhinitis. [Review]. Ear Nose Throat J 1989;68(3):207-10.
4. Small P, Black M, Frenkiel S. Effects of treatment with beclomethasone dipropionate in subpopulations of perennial rhinitis patients. J Allergy Clin Immunol 1982;70(3):178-82.
5. Blom HM, Godthelp T, Fokkens WJ, Kleinjan A, Holm AF, Vroom TM, et al. Mast cells, eosinophils and IgE-positive cells in the nasal mucosa of patients with vasomotor rhinitis. An immunohistochemical study. Eur Arch Otorhinolaryngol Suppl 1995;1(9):S33-9.
6. Blom HM, Severijnen LA, van Rijswijk JB, Mulder PG, Gerth van Wijk R, Fokkens WJ. The long-term effects of capsaicin aqueous spray on the nasal mucosa. Clin Exp Allergy 1998;28(11):1351-8.
7. Bagenstose SE, Bernstein JA. Treatment of chronic rhinitis by an allergy specialist improves quality of life outcomes. Ann Allergy Asthma Immunol 1999;83(6 Pt 1):524-8.
8. Sanico A, Togias A. Noninfectious, nonallergic rhinitis (NINAR): considerations on possible mechanisms. Am J Rhinol 1998;12(1):65-72.
9. Baraniuk JN, Kaliner MA. Neuropeptides and nasal secretion. J Allergy Clin Immunol 1990;86(4 Pt 2):620-7.
10. Fang SY, Shen CL. Neuropeptide innervation and neuroendocrine cells in allergic rhinitis and chronic hypertrophic rhinitis. Clin Exp Allergy 1998;28(2):228-32.
11. Lacroix JS, Kurt AM, Pochon N, Bretton C, Lunberg JM, Deshusses J. Neutral endopeptidase activity and concentration of sensory neuropeptide in the human nasal mucosa. Eur Arch Otorhinolaryngol 1995;252:465-468.
12. Wolf G, Anderhuber W, Hauser-Kronberger C, Saria A. [Treatment of nonspecific hyper-reflectory rhinopathy (vasomotor rhinitis) with capsaicin]. Laryngorhinootologie 1995;74(5):289-93.

13. Riechelmann H, Davris S, Bader D. [Treatment of perennial non-allergic rhinopathy with capsaicin]. Hno 1993;41(10):475-9.
14. Marabini S, Ciabatti PG, Polli G, Fusco BM, Geppetti P. Beneficial effects of intranasal applications of capsaicin in patients with vasomotor rhinitis. Eur Arch Otorhinolaryngol 1991;248(4):191-4.
15. Blom HM, van Rijswijk JB, Garrelds IM, Mulder PG, Timmermans T, Gerth van Wijk R. Intranasal capsaicin is efficacious in non-allergic, non-infectious perennial rhinitis. A placebo-controlled study. Clin Exp Allergy 1997;27(7):796-801.
16. Lacroix JS, Buvelot JM, Polla BS, Lundberg JM. Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy 1991;21(5):595-600.
17. Kitajiri M, Kubo N, Ikeda H, Sato K, Kumazawa T. Effects of topical capsaicin on autonomic nerves in experimentally-induced nasal hypersensitivity. An immunocytochemical study. Acta Otolaryngol Suppl 1993;500(2):88-91.
18. Mygind N, Weeke B. Allergic and vasomotor rhinitis: Clinical aspects. Copenhagen: Munksgaard; 1985.
19. Doty RL, Shaman P, Kimmelman CP, Dann MS. University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. Laryngoscope 1984;94(2 Pt 1):176-8.
20. Braat JP, Mulder PG, Fokkens WJ, Gerth van Wijk R, Rijntjes E. Intranasal cold dry air is superior to histamine challenge in determining the presence and degree of nasal hyperreactivity in nonallergic noninfectious perennial rhinitis. Am J Respir Crit Care Med 1998;157(6 Pt 1):1748-55.
21. Geppetti P, Tramontana M, Del Bianco E, Fusco BM. Capsaicin-desensitization to the human nasal mucosa selectively reduces pain evoked by citric acid. Br J Clin Pharmacol 1993;35(2):178-83.
22. Hilberg O, Pedersen OF. Acoustic rhinometry: recommendations for technical specifications and standard operating procedures. Rhinol Suppl 2000;16:3-17.
23. Phagoo SB, Watson RA, Pride NB. Use of nasal peak flow to assess nasal patency. Allergy 1997;52(9):901-8.

CHAPTER V

Inflammatory cells seem not to be involved in idiopathic rhinitis.

J.B. van Rijswijk, H.M. Blom, A. Kleinjan, P. G. H.Mulder,
E. Rijntjes and W. J. Fokkens

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ABSTRACT

Mucosal inflammatory cellular infiltrates are correlated with nasal complaints in symptomatic allergic rhinitis. Some authors suggest inflammation of a neurogenic or immunogenic nature as an underlying disorder for idiopathic rhinitis (IR). We looked at the possible involvement of inflammatory cells in the pathogenesis of IR. Nasal biopsies were taken from sixty-five IR patients with significant nasal complaints and from twenty healthy controls with no nasal complaints. Inflammatory cells were quantified using monoclonal antibodies directed against lymphocytes, antigen-presenting cells, eosinophils, macrophages, monocytes, mast cells and other IgE-positive cells. No significant differences were found, for any cell, between IR patients and controls. We conclude that inflammatory cells do not seem to play an important role in this meticulously characterised group of IR patients.

INTRODUCTION

Idiopathic rhinitis (IR) is a diagnosis by exclusion. This disorder probably represents a heterogeneous group of pathophysiological conditions. This implies that the study group needs to be meticulously characterised. In a group of non-atopic patients with nasal complaints, we excluded all patients with systemic, allergic, medical and anatomical disorders that could explain complaints of rhinorrhea, sneezing and/or nasal obstruction. This group with unexplained nasal complaints was then homogenised on the basis of a daily record chart on which patients had to reach a minimum symptom score. The minimum was set using as a basis the definition of rhinitis put forward by Mygind(Mygind and Weeke, 1985). In affected patients, periods of nasal discharge, sneezing and congestion had to persist for an average of at least 1-hour per day on at least five days during a period of fourteen days.

The proposed pathophysiological mechanisms for IR include a chronic inflammatory disorder of antigenic (local allergy) or neurogenic nature(Philip and Togias, 1995, Carney and Jones, 1996, Shatkin et al., 1994). A pivotal characteristic in the pathophysiological concept of inflammation is an influx of inflammatory cells in the affected tissue. In symptomatic allergic rhinitis, an increase of inflammatory cells has been observed in the nasal mucosa(Bentley et al., 1992, Fokkens et al., 1990, Braunstahl et al., 2001). We showed that cellular infiltrates (eosinophils, mast cells and IgE positive cells) were not significantly different in a group of IR patients compared to healthy controls (Blom et al., 1995). To ascertain the significance of inflammation, we also need to know whether regulatory cells (lymphocytes and antigen presenting cells) are involved in IR.

In this study, we examined nasal biopsies from 65 symptomatic IR patients and 20 healthy controls without nasal complaints. The cell densities of CD1, CD3, CD4, CD8, CD14, CD25, CD68, chymase, tryptase, IgE and BMK13 were studied in both layers of the nasal mucosa.

MATERIALS AND METHODS

Subjects

Patients were studied in the outpatient ENT departments of the Leyenburg Hospital in The Hague and the Erasmus Medical Centre Rotterdam University Hospital in Rotterdam, The Netherlands.

Patients were admitted to the study if they had a history of nasal complaints such as nasal obstruction, sneezing, and rhinorrhea for a period of over 1 year which could not be attributed to allergic rhinitis, nasal or paranasal sinus infection, anatomical disorders affecting nasal function, pregnancy or lactation, systemic disorders and/or the use of medication affecting nasal function (Table 1). Patients with nasal polyps were excluded, since they may belong to a different pathophysiological group and their polyps may contribute to a higher symptom score for nasal blockage and/or rhinorrhea.

In affected patients, periods of nasal discharge, sneezing and congestion scored using a daily record chart (DRC, table 2) had to persist for an average of at least 1h per day on at least 5 days during a period of 14 days. Sixtyfive patients participated under conditions of informed consent (male/female: 32/33); the mean age was 34 years (range: 17-62). The ethnic origin of the patients was: 56 Caucasian, 6 Asian, 2 Negroid and 1 Oriental. The control group consisted of twenty healthy volunteers (male/female: 11/9); mean age 36 years (range: 18-62), 16 Caucasian, 3 Oriental, and 1 Asian, without nasal complaints or nasal abnormalities on ENT examination, a negative skin prick test for the common inhalation allergens and a negative Phadiatop (Pharmacia, Uppsala, Sweden). Patients and healthy controls were biopsied once. Procedures were approved by the local Medical Ethics committees.

Nasal biopsies

At the time of the biopsy, all patients had nasal complaints, as confirmed by their daily record charts. Controls did not suffer from nasal complaints. After randomisation of the biopsy side, a biopsy of nasal mucosa was taken from the lower edge of the inferior turbinate, about 2 cm posterior to the front edge, using a Gerritsma forceps with a cup diameter of 2.5 mm (Fokkens et al., 1988). Local anaesthesia was obtained by placing a cotton-wool carrier

Table 1. Selection criteria for idiopathic rhinitis

Inclusion criteria

- Age between 16 and 64 years.
- Negative skin prick test: house dust mite, tree pollen mix, grass pollen mix, mugwort, alternaria, aspergillus, cladosporium, penicillum, dog, cat, parakeet, rabbit, hamster, horse, guinea pig. (ALK-Diephuis, Holland)
- Negative Phadiatop (Pharmacia, Uppsala, Sweden)
- Symptoms for more than 1 year.
- Periods of nasal discharge, sneezing and congestion for an average of at least 1 h per day on at least 5 days during a period of 14 days.

Exclusion criteria

- The use of systemic or inhaled corticosteroids within the previous month.
- Use of inhaled sodium cromoglycate or nedocromil sodium within the previous month.
- Use of astemizole within the previous month.
- Inability of the patient to stop taking medication affecting nasal function.
- A serious and/or unstable disease.
- Nasal surgery within the previous 6 weeks.
- Nasal polyps or a history of nasal polyps.
- Significant anatomical abnormalities affecting nasal function.
- Nasal or paranasal sinus infection (abnormal sinus X-ray).
- Pregnancy or lactation
- Abnormal findings at physical examination.
- Abnormal laboratory results for:

blood: Na, K, Ca, total protein, albumin, urea, creatinine, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gammaglutamyl transpeptidase, haemoglobin, red blood cell count, plasma cell volume, mean corpuscular volume, platelets, total white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils.

urine: blood, protein, glucose.

Table 2. Specimen of the daily record card for defining nasal symptoms in patients with idiopathic rhinitis.

Possible scores on the daily record chart	
Nasal blockage:	0 = absent
(not being able to breathe freely through the nose)	1 = between 0-1 h per half day
Clear nasal discharge: (runny nose)	2 = between 1-2 h per half day
	3 = more than 2 h per half day
Sneezing	0 = absent
Coughing	1 = less than 5 periods per half day
	2 = between 5-10 periods per half day
	3 = more than 10 periods per half day
Mucus production:	0 = absent
(yellow, green or brown)	1 = present

with 50 mg of cocaine and one drop of adrenaline (1:1000) under the inferior turbinate without touching the biopsy site. The specimens were embedded in Tissue-Tek II O.C.T. compound and frozen immediately.

Staining procedures

Monoclonal antibodies (mAb) directed against CD1, CD3, CD4, CD8, CD14, CD25, CD 68, chymase, tryptase, IgE, and BMK13 (table 3) were used together with the supersensitive immunoalkaline phosphatase (ss-AP) method. Six micron thick sections of nasal mucosa were cut on a cryostat (Jung Frigocut 2800E/20/40), transferred to poly-L-lysine-coated microscope slides, dried, and fixed in acetone for 10 minutes at room temperature. They were then

Table 3. Monoclonal antibodies used to study muscosal biopsies in patients with idiopathic rhinitis and controls.

Antibody	Specificity	Titer	Source
CD1	OKT6	1:100	Dept. Immunology, Erasmus University, Rotterdam, The Netherlands (NL)
CD3 CD4 CD8 CD25	leu4 leu3 leu2 IL2-r	1:25 1:50 1:100 1:150	BDH, Dorset, UK
B7 G3	Chymase Tryptase	1:100 1:250	Chemicon, Temecula, Calif, USA
BMK13	MBP	1:200	Sanbio, Uden, NL
CD14 anti-IgE	mon/1 IgE	1:20 1:250	Central laboratory of the Netherlands Red Cross Blood Transfusion service (CLB), Amsterdam, NL
CD68	KI-M6	1:50	Behring, Marburg, Germany

rinsed in phosphate-buffered saline (PBS, pH 7.2), placed in a half-automatic stainer (Sequenza, Shandon), incubated with 2 % bovine serum albumin in PBS for 10 minutes and incubated with normal goat serum (CLB, Amsterdam, The Netherlands) for 10 minutes. The slides were then incubated with the mAb for 30 minutes at room temperature.

The sections were then rinsed again in PBS for 5 minutes and incubated for 30 minutes with a biotinylated goat anti-mouse (1:50) immunoglobulin antiserum, rinsed successively in PBS, incubated with strept Avidin AP (1:50) (Biogenics, Klinipath, Duiven, Netherlands) for 30 minutes at room temperature, rinsed in PBS and TRIS buffer (pH 8.0), and incubated for 30 minutes with a new fuchsin substrate (Chroma, Kongen, Germany). Finally, the sections were rinsed with distilled water, counterstained with Gills hematoxylin and mounted in glycerin-gelatin. Control staining was performed

by substitution with PBS and incubation with an irrelevant mAb of the same subclass.

Light-microscopic evaluation

Stained cells were counted in two sections of each biopsy specimen. The epithelium and lamina propria were evaluated separately. The total surface area of the sections and their main parts (i.e. the epithelium and the lamina propria) were estimated using the Kontron Image Analysis System Videoplan. The number of cells/mm^2 was calculated for the epithelium and the lamina propria.

Statistical analysis.

The non-parametric Mann-Whitney test was used to compare the difference in cell counts between the two groups. A p-value < 0.05 was considered to indicate a significant difference. In order to have some idea of the magnitude of a Type II error in this study, the 97.5 upper confidence limit of the mean difference between patients and controls was calculated after ln-transformation of the cell counts. The ln-transformation compensates for the positive skewness so as to justify parametric inference more properly. The antilog of this upper confidence limit divided by the antilog of this mean difference gives the maximum ratio between the larger and the smaller median that would still be accepted at the 5% level (2-sided), given the non-significantly different observed medians in patients and controls. We call this ratio (which by definition is larger than one) the smallest detectable ratio of medians between the two groups for a particular variable in this study. The more lack of power (i.e., the larger the Type II error), the higher this ratio will be. It is assumed here that the distribution of the variable considered is lognormal so that the geometric mean coincides with the median.

For instance, if the smaller of both medians for CD3 epithelium equals 512 in the control group and the calculated smallest detectable ratio of medians between the two goups is 1.57, then the patient group would be significantly different from the control group if the median is at least equal to 1.57 x 512 = 804.

RESULTS

Biopsy specimen

The sections of the nasal mucosa had an average surface area of 1.6 mm^2 and usually had a lining of ciliated columnar epithelium with or without goblet cells and/ or partially stratified cuboidal epithelium. The lamina propria usually consisted of a looser subepithelial cell-rich layer with most of the mucous glands and a deeper collagenous cell-poor layer. All sections were sufficiently deep to assess both layers. The sections were generally of good quality. It was not possible to evaluate two biopsy specimens. One exclusion was made because of an artefact resulting from defrosting of the specimen and the other specimen was displaced. The mAb-ss-AP staining showed red cells against a blue counterstained background. Biopsy specimens from 2 of the 65 patients showed substantial numbers of eosinophils, mast cells and IgE-positive cells.

T-lymphocytes

These small round cells were abundantly present in the epithelium and in the lamina propria. Sometimes, clusters of T-cells (500-1000 cells) were found in the lamina propria. There was no difference between the groups in terms of the presence of these clusters.

The numbers of CD3, CD4, CD8, and CD25 positive cells/mm^2 are shown in table 4. As can be seen, hardly any IL-2 receptor (CD25) positive cells were found in either layer of the nasal mucosa. If there were any differences between the two groups at all, they were not statistically significant. The smallest detectable ratios of medians between the groups were, respectively: CD3 epithelium (EP) 1.57, CD3 lamina propria (LP) 1.47, CD4 EP 1.78, CD4 LP 1.5, CD8 EP 1.88, CD8 LP 1.73, CD25 EP 2.63, CD25 LP 2.38.

Langerhans cells

This large dendritic cell was found mostly in the epithelium. Only a few were present in the lamina propria. The numbers of CD1-positive cells are shown in table 4. No significant differences were found. The smallest detectable ratios of medians between the groups were, respectively: CD1 EP 1.86, CD1 LP 2.20.

Table 4. Median (25th and 75th percentile of positive cells/mm^2 in epithelium and lamina propria of the nasal mucosa.

Cell type	Controls	Patients	p-value
	Median (25 %-75%)	Median (25%-75%)	
Epithelium			
CD1	48 (15-130)	54 (15-110)	0.82
CD3	512 (299-867)	630 (347-1079)	0.27
CD4	545 (341-755)	424 (223-584)	0.18
CD8	305 (173-431)	446 (163-762)	0.11
CD14	310 (130-497)	215 (179-316)	0.68
CD25	8 (0-30)	0 (0-25)	0.43
BMK13	0 (0-0)	0 (0-0)	0.60
Tryptase	0 (0-4)	0 (0-4)	0.88
Chymase	0 (0-0)	0 (0-8)	0.06
IgE	0 (0-0)	0 (0-28)	0.30
CD68	165 (89-293)	214 (136-378)	0.06
Lamina propria			
CD1	3 (1-8)	5 (1-13)	0.54
CD3	678 (486-832)	552 (300-872)	0.31
CD4	464 (181-885)	426 (259-611)	0.65
CD8	269 (160-345)	295 (147-476)	0.51
CD14	232 (143-367)	196 (161-271)	0.58
CD25	7 (2-58)	3 (0-13)	0.30
BMK13	0 (0-0)	0 (0-3)	0.18
Tryptase	65 (41-71)	69 (38-97)	0.30
Chymase	54 (47-71)	63 (46-100)	0.35
IgE	8 (2-62)	22 (4-64)	0.67
CD68	145 (74-195)	152 (101-250)	0.30

Macrophages and monocytes
The CD68 positive cells were large cells with a bright staining cytoplasm. These cells were found to be equally distributed in both layers, as was CD14. The numbers of CD68 and CD14 cells are shown in table 4. No significant differences were found. The smallest detectable ratios of medians between the groups were, respectively: CD14 EP 1.68, CD14 LP 1.55, CD68 EP 1.44, CD 68 LP 1.52.

Mast cells and other IgE-positive cells
The chymase and tryptase and IgE-positive cells were found mainly in the lamina propria. The numbers are shown in table 4. No significant differences were found. The smallest detectable ratios of medians between the groups were, respectively: anti-IgE EP 4.22, anti-IgE LP 3.17, tryptase EP 2.62, tryptase LP 1.57, chymase EP 2.47, chymase LP 1.61.

Eosinophils
The numbers of BMK13 positive cells found in the nasal mucosa of both patients and controls were negligible. The numbers are shown in table 4. No significant differences were found. The smallest detectable ratios of medians between the groups were, respectively: BMK13 EP 1.96, BMK13 LP 2.21.

DISCUSSION

Wolf suggested that IR could be the result of an "over-active" non-adrenergic non-cholinergic system(Wolf, 1988). Stimulation of sensory neurons results in rhinorrhea, nasal blockage and sneezing(Baraniuk, 1992). Sensory neural stimulation may produce these effects either through a central neural reflex, associated with efferent parasympathetic neurotransmission, or via anti-dromic release of neuropeptides from sensory neurons (Lundblad et al., 1983). This hypothesis was corroborated by the findings of Lacroix, who reported an increased concentration of neuropeptides in a group of chronic nonallergic rhinitis patients (Lacroix et al., 1992), improvement of symptoms by local treatment of capsaicin giving a 50% reduction in CGRP-like immunoreactivity(-LI) content in nasal biopsies (Lacroix et al., 1991), and a corre-

lation between symptom intensity and CGRP-LI concentration in nasal mucosa (Lacroix et al., 1995).
An increase of proinflammatory neuropeptides may result in a stimulation of T-cell proliferation, stimulation of mast cells, macrophages and eosinophils, and chemoattraction of eosinophils and neutrophils(Joos et al., 1995). Substance P can increase the percentage of neutrophils recovered from nasal lavage (Braunstein et al., 1994). Capsaicin, a specific activator of sensory nerve endings, induces a neurogenic inflammation, with an influx of inflammatory cells in nasal lavage after a single provocation (Philip et al., 1996).
Another theory concerning the pathogenesis of IR is that of a local, occult allergy (Carney et al., 2001, Powe et al., 2001, Shatkin et al., 1994). The diagnosis of IR is made by exclusion. An allergy test is not 100% sensitive and systemic manifestations of atopic disease, such as a positive skin prick test or RAST, may be missed because the nose is a small shock organ. In seasonal or perennial allergic rhinitis, increased numbers of inflammatory cells, such as Langerhans cells, IgE positive cells and eosinophils, can be found in the nasal mucosa as a sign of inflammation (Bentley et al., 1992, Fokkens et al., 1990, Braunstahl et al., 2001).
By strict selection and by using a complaint threshold value, we tried to achieve a homogenous group of patients. The 2 patients of the total of 65 patients with negative allergy tests who had a substantial typical cellular allergic infiltrate in the nasal mucosa were classified as possible sufferers from an occult local allergy and or non-allergic rhinitis with eosinophil syndrome (NARES) (Mikaelian, 1989). This would mean a maximum prevalence of three percent of occult allergy/NARES in this group that can be discerned by nasal biopsies. No other signs of inflammation were found in this IR patient group.
This contrasts with a recent study of Powe et al. who found significantly more nasal mucosa mast cells and eosinophils in a group of IR (and allergic rhinitis) patients compared to a group of normal individuals(Powe et al., 2001). They examined whole, full-length, full-thickness concha inferior specimens resected under general anaesthesia.
The difference in study outcome may be explained by a more severe pathology in the IR group of Powe et al. warranting total turbinectomy. Another explanation could be the difference in biopsy size (average surface area of

1.6 mm^2 in our study). Nasal cellular infiltrates show a focal localisation of cell populations which can be better averaged in larger biopsies.
It may also be the case that our IR patient group contains significantly fewer NARES patients (2 of the 65) compared to the patient group studied by Powe et al. The reason for this could be the fact that a Dutch rhinitis patient will not be sent to the ENT department before being treated with local corticosteriods by his or her general practitioner (Lundblad et al., 2001). In addition, as might be expected, it seems that NARES patients and or patients with an occult local allergy form an IR subgroup which responds well to nasal corticosteroids (Small et al., 1982).
The data presented concurs with the data from Sanico, who was unable to find an increased responsiveness to capsaicin in a group of 8 non-allergic rhinitis patients. He therefore argued against a central role for capsaicin sensitive nerves (pivotal in the concept of neurogenic inflammation) in the pathophysiology of IR (Sanico et al., 1998).
The question then arises as to whether this immunohistochemical evaluation method is sensitive enough to detect significant differences between the groups. The calculated ratios between the geometric means of both groups indicating threshold significance at the 5% level are within the range found in patients with chronic allergic rhinitis (Godthelp et al., 1996, Fokkens et al., 1990). In these studies, which compare symptomatic allergic patients to asymptomatic controls, cellular differences between patients and controls were indeed found, while the distribution of the number of immunocompetent cells/mm^2 was in the same order of magnitude as in this IR study. We therefore think it is justified to assume that if significant mucosal inflammation was present, we would have detected it. The lack of differences in cell numbers does not exclude a functional cellular involvement. However, in two other studies, we failed to ascertain a relation between the number of immunocompetent cells and nasal complaints in IR patients (Blom et al., 1998, Blom et al., 1997a). A significant reduction of immunocompetent cells in the nasal mucosa of IR patients treated with nasal steroids (fluticasone aqueous nasal spray) was not accompanied by a reduction in nasal complaints (Blom et al., 1997a) and, inversely, a significant reduction in nasal complaints in a group of IR patients treated with topical capsaicin aqueous nasal spray was not accompanied by a change in inflammatory mediators (Blom et al., 1997b) or a reduction in the numbers of inflammatory cells (Blom et al., 1998).

Given the above, we conclude that inflammatory cells do not seem to play an important role in this meticulously characterised group of IR patients.

REFERENCES

Baraniuk, J. N. (1992). Sensory, parasympathetic, and sympathetic neural influences in the nasal mucosa. J Allergy Clin Immunol, 90, 1045-50.

Bentley, A. M., Jacobson, M. R., Cumberworth, V., Barkans, J. R., Moqbel, R., Schwartz, L. B., Irani, A. M., Kay, A. B. and Durham, S. R. (1992). Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. J Allergy Clin Immunol, 89, 877-83.

Blom, H. M., Godthelp, T., Fokkens, W. F., Kleinjan, A., Mulder, P. G. M. and Rijntjes, E. (1997a). The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with a non-allergic non-infectious perennial rhinitis. J Allergy Clin Immunol, 100, 739-47

Blom, H. M., Godthelp, T., Fokkens, W. J., Kleinjan, A., Holm, A. F., Vroom, T. M. and Rijntjes, E. (1995). Mast cells, eosinophils and IgE-positive cells in the nasal mucosa of patients with vasomotor rhinitis. An immunohistochemical study. Eur Arch Otorhinolaryngol Suppl, 1, S33-9.

Blom, H. M., Severijnen, L. A., van Rijswijk, J. B., Mulder, P. G., Gerth van Wijk, R. and Fokkens, W. J. (1998). The long-term effects of capsaicin aqueous spray on the nasal mucosa. Clin Exp Allergy, 28, 1351-8.

Blom, H. M., Van Rijswijk, J. B., Garrelds, I. M., Mulder, P. G., Timmermans, T. and Gerth van Wijk, R. (1997b). Intranasal capsaicin is efficacious in non-allergic, non-infectious perennial rhinitis. A placebo-controlled study. Clin Exp Allergy, 27, 796-801.

Braunstahl, G. J., Overbeek, S. E., Fokkens, W. J., Kleinjan, A., McEuen, A. R., Walls, A. F., Hoogsteden, H. C. and Prins, J. B. (2001). Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. Am J Respir Crit Care Med, 164, 858-65.

Braunstein, G., Buvry, A., Lacronique, J., Desjardins, N. and Frossard, N. (1994). Do nasal mast cells release histamine on stimulation with substance P in allergic rhinitis? [see comments]. Clin Exp Allergy, 24, 922-9.

Carney, A. S., Hooi, D., Powe, D. G., Huskisson, R. S. and Jones, N. S. (2001). Autoanti-IgE antibodies in patients with allergic and idiopathic rhinitis. Clin Otolaryngol, 26, 298-301.

Carney, A. S. and Jones, N. S. (1996). Idiopathic rhinitis: idiopathic or not? Clin Otolaryngol, 21, 198-202.

Fokkens, W. J., Holm, A. F., Rijntjes, E., Mulder, P. G. and Vroom, T. M. (1990). Characterization and quantification of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. Int Arch Allergy Appl Immunol, 93, 66-72.

Fokkens, W. J., Vroom, T. M., Gerritsma, V. and Rijntjes, E. (1988). A biopsy method to obtain high quality specimens of nasal mucosa. Rhinology, 26, 293-5.

Godthelp, T., Holm, A. F., Fokkens, W. J., Doornenbal, P., Mulder, P. G., Hoefsmit, E. C., Kleinjan, A., Prens, E. P. and Rijntjes, E. (1996). Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol, 97, 800-11.

Joos, G. F., Germonpre, P. R. and Pauwels, R. A. (1995). Neurogenic inflammation in human airways: is it important? Thorax, 50, 217-9.

Lacroix, J. S., Buvelot, J. M., Polla, B. S. and Lundberg, J. M. (1991). Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy, 21, 595-600.

Lacroix, J. S., Kurt, A. M., Pochon, N., Bretton, C., Lunberg, J. M. and Deshusses, J. (1995). Neutral endopeptidase activity and concentration of sensory neuropeptide in the human nasal mucosa. Eur Arch Otorhinolaryngol, 252, 465-468.

Lacroix, J. S., Pochon, N. and Lundberg, J. M. (1992) In Secondary*Increased concentration of sensory neuropeptide in the nasal mucosa from patients with non-allergic chronic rhinitis*, Vol. II Monduzzi Editore S.p.A., Italy, Sorrento, Italy, pp. 59-63.

Lundblad, L., Saria, A., Lundberg, J. M. and Anggard, A. (1983). Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons. Acta Otolaryngol, 96, 479-84.

Lundblad, L., Sipila, P., Farstad, T. and Drozdziewicz, D. (2001). Mometasone furoate nasal spray in the treatment of perennial non-allergic rhinitis: a nordic, multicenter, randomized, double-blind, placebo-controlled study. Acta Otolaryngol, 121, 505-9.

Mikaelian, A. J. (1989). Vasomotor rhinitis. [Review]. Ear Nose Throat J, 68, 207-10.

Mygind, N. and Weeke, B. (1985) *Allergic and vasomotor rhinitis: Clinical aspects,* Munksgaard, Copenhagen.

Philip, G., Sanico, A. M. and Togias, A. (1996). Inflammatory cellular influx follows capsaicin nasal challenge. Am J Respir Crit Care Med, 153, 1222-9.

Philip, G. and Togias, A. G. (1995). Nonallergic rhinitis. Pathophysiology and models for study. Eur Arch Otorhinolaryngol Suppl, 1, S27-32.

Powe, D. G., Huskisson, R. S., Carney, A. S., Jenkins, D. and Jones, N. S. (2001). Evidence for an inflammatory pathophysiology in idiopathic rhinitis. Clin Exp Allergy, 31, 864-72.

Sanico, A. M., Philip, G., Proud, D., Naclerio, R. M. and Togias, A. (1998). Comparison of nasal mucosal responsiveness to neuronal stimulation in non-allergic and allergic rhinitis: effects of capsaicin nasal challenge. Clin Exp Allergy, 28, 92-100.

Shatkin, J. S., Delsupehe, K. G., Thisted, R. A. and Corey, J. P. (1994). Mucosal allergy in the absence of systemic allergy in nasal polyposis and rhinitis: a meta-analysis. Otolaryngol Head Neck Surg, 111, 553-6.

Small, P., Black, M. and Frenkiel, S. (1982). Effects of treatment with beclomethasone dipropionate in subpopulations of perennial rhinitis patients. J Allergy Clin Immunol, 70, 178-82.

Wolf, G. (1988). [New aspects in the pathogenesis and therapy of hyperreflexive rhinopathy].

CHAPTER VI

The Long-term Effects of Capsaicin Aqueous Spray on the Nasal Mucosa.

H. M. Blom, L. A. W. F. M. Severijnen, J. B. van Rijswijk,
P. G. H. Mulder, R. Gerth van Wijk and W. J. Fokkens.

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ABSTRACT

Background: Capsaicin has been shown previously to reduce nasal complaints in patients with idiopathic rhinitis. Proposed pathophysiological mechanisms for idiopathic rhinitis include a chronic inflammatory disorder of an antigenic or neurogenic nature as well as the possibility of a functional neuronal disorder. We hypothesized that the beneficial effect of capsaicin might be the result of a down-regulation of inflammation (by a reduction of inflammatory cells) or through modulation of neural tissue density.
Methods: Patients were treated with either a placebo or capsaicin spray solution delivering 0.15 mg of capsaicin per nostril once every second or third day for a total of seven treatments. Both sides were treated each visit. Biopsies were taken before and 2 weeks, 3 months and 9 months after the treatment period. Immunohistochemical staining of the biopsy specimen was performed to ascertain the effect of treatment on immunocompetent cell densities (quantitative) and neural tissue densities (semi-quantitative) in the nasal mucosa.
Results: Nasal complaints were significantly reduced in the capsaicin-treated group. The number of CD1+, CD25+, CD3+, CD68+, BMK13+, IgE+, tryptase+, and chymase+ cells did not significantly differ between capsaicin and placebo group. No significant differences between both groups were found in pan-neurogenic staining of nasal mucosa using neurofilament and synaptophysine.
Conclusion: Capsaicin aqueous nasal spray has previously been shown to reduce nasal complaints without affecting cellular homeostasis or overall neurogenic staining up to 9 months after treatment. Immunocompetent cells are not involved in idiopathic rhinitis.

INTRODUCTION

In a double-blind placebo-controlled study we recently demonstrated that capsaicin is highly effective in controlling idiopathic rhinitis (1). A long-lasting relief in symptoms was obtained for at least 9 months.
Capsaicin is the pungent agent in red peppers. Its mode of action is well documented in rodents, where it affects mainly the thin, unmyelinated sen-

sory nerve fibres. It causes initial stimulation (with release of endogenous neuropeptides), followed by desensitization to capsaicin and other sensory stimuli (2). With higher doses, long-term functional or even morphological ablation of the thin sensory neurons occurs (3). In humans, the effect of capsaicin has not been fully documented (4). Moreover, the pathophysiological mechanism for idiopathic rhinitis is not understood. Proposed mechanisms include a chronic inflammatory disorder of an antigenic or neurogenic nature, or a functional neuronal disorder (5,6).
To study whether capsaicin reduces inflammation or modulates nasal neuronal tissue densities we performed a nasal biopsy study in 24 patients with idiopathic rhinitis. Cells were quantified per square millimetre and the sections stained with neuronal markers were scored semi-quantitatively for morphometric changes.

MATERIALS AND METHODS

Subjects
Patients were admitted to the study if they had a history of nasal complaints such as nasal obstruction, sneezing, and rhinorrhea for a period of over 1 year which could not be attributed to allergic rhinitis, nasal or paranasal sinus infection, anatomical disorders affecting nasal function, pregnancy or lactation and/or systemic disorders (7,8). They were non-smokers not using medication affecting nasal function. Patients with nasal polyps were excluded, since they may belong to a different pathophysiological group and their polyps may contribute to a higher symptom score for nasal blockage and/or rhinorrhea.

Study design
Thirty-five patients with the diagnosis of idiopathic rhinitis scored nasal blockage, clear discharge, sneezing and coughing using a four point scale; mucus production (absent or present) was noted for a period of 2 weeks using a daily record card (1,7,8). Mucus production or coughing were used as indicators of upper airway infection. If present they led to exclusion of the pa-

tient. Patients were included in this study if periods of clear nasal discharge, sneezing and nasal blockage persisted for an average of at least 1 h/day for at least 5 days during a period of 14 days. The duration of complaints during the day was used as the prime criterion for further study. Twenty-five of the 35 patients were found eligible for our study and participated under conditions of informed consent (male/female: 16/9); mean age was 36 years (range: 18-60 years). One of the 14 capsaicin patients could not continue after three capsaicin applications because of influenza with fever.
Procedures were approved by the local medical ethics committee.
Patients were randomized in a double-blind placebo-controlled fashion and treated with placebo (11 persons) or capsaicin (14 persons). A total of seven treatments over a period of 2 weeks were given.

Treatment procedure
The nose was decongested with xylometazolinehydrochloride 0.1% and anaesthetized with lidocaine base-spray (100 mg/mL). Capsaicin aqueous nasal spray (0.15 mg) or placebo were instilled in each nostril (1). The application of Xylocaine® spray in the nasal airway was immediately followed by a painful sensation that was described by all subjects as most unpleasant. Patients did not complain of irritation of nose and lips during or after capsaicin/placebo application. At every visit the subjects rated nasal symptoms on a visual analogue scale (0-10 cm; 0 represented absence of symptoms and 10 represented high intensity of symptoms). Daily record card scoring was continued for up to 2 weeks after treatment (1).
Nasal biopsies were taken four times: at the run-in period, and 2 weeks, 3 months and 9 months after the treatment period (Fig. 1). After randomization of the biopsy side, specimens of nasal mucosa were taken from the lower edge of the inferior turbinate, about 2 cm posterior to the front edge, using a Gerritsma forceps with a cup diameter of 2.5 mm (9). Local anaesthesia was obtained by placing a cotton-wool carrier with 50 mg of cocaine and one drop of adrenaline (1:1000) under the inferior turbinate without touching the biopsy site. The specimens were embedded in Tissue-Tek II OCT (Sakura Finetek Europe BV, Zoeterwoude, The Netherlands) compound and frozen immediately.
Blood: (sodium, potassium, calcium, total protein, albumin, urea, creatinine,

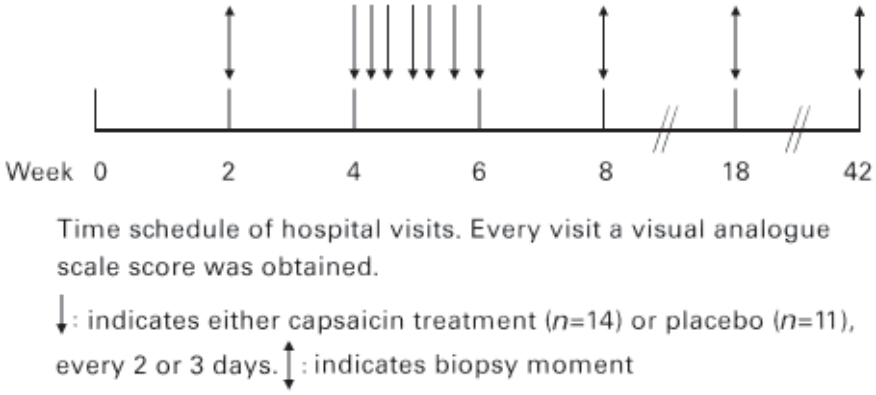


Fig. 1. Study design during the entire study period patients scored their nasal complaints such as nasal blockage, clear discharge, sneezing, coughing and mucus production on a daily record card. After the first 2 weeks (0-2, run-in) patients with periods of nasal blockage, clear nasal discharge, and sneezing persisted for an average of at least 1 h/day for at least 5 days during the 14 day period. Mucus production and coughing were used as upper airway indicators. If present they led to exclusion of the patient. A biopsy was taken in included patients. Weeks 2-4 were used to allow healing of the nasal mucosa before treatment. During weeks 4-6 a total of seven treatments were given. During the evaluation period (weeks 6-42) three nasal biopsies were taken.

bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gammaglutamyl transpeptidase, haemoglobin, red blood cell count, plasma cell volume, mean corpuscular volume, platelets, total white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils) and urine: (blood, protein, glucose) samples were taken during visits one and nine to monitor changes during therapy.

Staining procedures

Monoclonal antibodies (mAb) directed against synaptophysine, neurofilament, CD1, CD3, CD25, CD68, IgE, MBP, chymase and tryptase (Table 1) were used together with the super sensitive immunoalkaline phoshatase (ss-APAAP) method. Sections of nasal mucosa were cut at 6 μm on a cryostat (Jung Frigocut 2800E/20/40), transferred to poly L-lysine-coated microscope slides, dried

and fixed in acetone for 10 min at room temperature. They were next rinsed in phosphate-buffered saline (PBS, pH 7.2), placed in a half-automatic stainer (Sequenza, Shandon, Amsterdam, The Netherlands) and incubated with normal goat serum (CLB, Amsterdam, The Netherlands) for 10 min. Following this the slides were incubated with the mAb for 60 min at room temperature. The sections were then rinsed again in PBS for 10 min and incubated for 30 min with a goat antimouse (1:50) biotin, rinsed successively in PBS, incubated with streptavidin alkaline phophatase supersensitive (1:50) (Biogenex, Klinipath, Duiven, The Netherlands) for 30 min at room temperature, rinsed in PBS and TRIS buffer (pH 8.5), and incubated for 30 min with a new fuchsin substrate (Chroma, Kongen, Germany). Finally, sections were rinsed with distilled water, counter-stained with Gill's haematoxylin and mounted in glycerine-gelatine. Control staining was performed by substitution with PBS and incubation with an irrelevant mAb of the same subclass.

Light-microscopic evaluation

Stained cells were quantified ('blinded') in two sections of each biopsy specimen. The epithelium and lamina propria were evaluated separately. The total surface area of a section and its main parts (i.e. the epithelium and the lamina propria) were estimated with the use of the Kontron Image Analysis System Videoplan (Zeiss, Weesp, The Netherlands). The number of cells/

Table 1. Monoclonal antibodies used to study nasal biopsy specimen

Antibody	Specificity	Titre	Source
OKT6	CD1	1 : 100	Dept. Immunology, Erasmus University, Rotterdam
leu4	CD3	1 : 25	BDH, Dorset. UK
KIM-6	CD68	1 : 100	Behring, Rijswijk, NL
2F11	Neurofilament	1 : 50	Sanbio, Uden, The Netherlands
Sy38	Synaptophysine	1 : 20	Dakopatts, ITK, Uithoorn, NL
	IgE	1 : 250	Central laboratory of the Netherlands Red Cross Blood Tranfusion service (CLB), Amsterdam, NL
IL-2r	CD25	1 : 150	BDH, Dorset. UK
BMK13	MBP	1 : 200	Sanbio, Uden, The Netherlands
B7	Chymase	1 : 100	Chemicon, Temecula, Calif. USA
G3	Tryptase	1 : 250	Chemicon, Temecula, Calif. USA

mm2 was calculated for the epithelium and the lamina propria. The intensity, number and dimensions (width and length) of neuronal staining was semi-quantified by three separate observers. Biopsies were ranked 1-24 by continuously comparing the biopsies amongst another until all were ranked, by each separate observer. In practice a section would be taken at random, evaluated and put down. The next section would be taken at random and graded for stronger or weaker staining compared with the previous section. The next section would be stronger, weaker, or in between the two previous sections. At the end all sections would be 'on the table' and the weakest stained section would receive rank 1 and the strongest stained section would receive rank 24.

Statistical analysis

The non-parametric Mann-Whitney U-test was used to compare the differences in cell counts between the groups. A P-value < 0.05 was considered to indicate a significant difference. The Spearman rank correlations between changes in cell numbers and the changes in the visual analogue scale scores per randomization group were calculated. For the interobserver variation, the rank correlation between the rankings of any two observers was calculated per visit for synaptophysine and neurofilament. Differences in ranking between any two observers were also calculated. The mean rank averaged over the three observers was used to compare the two treatment groups per visit, using the Mann-Whitney U-test.

RESULTS

Biopsies

General description

The sections of the nasal mucosa had an average surface area of 2.0 mm^2 and usually showed a lining of ciliated columnar epithelium with or without goblet cells and/or partially stratified cuboidal epithelium. The lamina propria consisted usually of a looser subepithelial cell-rich layer with mucous glands and a deeper collagenous cell-poor layer. All sections were sufficiently deep to assess both layers. The sections were generally of good quality. No structural

Table 2a.

Epithelium	Run in		2 weeks		18 weeks		42 weeks	
Treatment	*plac*	*caps*	*Plac*	*caps*	*Plac*	*caps*	*Plac*	*caps*
CD1	379	341	451	447	275	355	230	350
25%	346	159	221	269	211	261	93	100
75%	846	425	970	791	591	529	278	621
CD3	1457	624	943	821	732	1340	1244	1339
25%	561	438	378	677	572	737	762	301
75%	2105	1432	1049	1557	1013	2992	1955	2667
CD25	84	34	59	86	52	50	44	36
25%	53	15	29	32	16	30	18	19
75%	128	92	67	176	182	195	64	129
CD68	455	578	512	587	480	1037	595	487
25%	228	340	334	379	305	472	421	377
75%	1934	800	709	856	1440	2071	875	1842
BMK 13	8	3	25	9	9	7	13	3
25%	1	2	0	2	0	0	0	0
75%	65	22	29	110	67	152	56	37
Tryptase	11	6	11	25	18	27	15	13
25%	0	4	0	0	0	11	0	0
75%	26	17	56	115	109	100	51	45
Chymase	0	3	16	12	7	15	14	36
25%	0	0	0	0	0	2	3	0
75%	8	56	26	61	18	42	19	105
IgE	74	123	57	36	153	56	29	20
25%	9	6	0	3	7	2	0	0
75%	311	206	420	160	182	269	344	185

Table 2. Median cell numbers and 25th and 75th percentiles for capsaicin and placebo treatment at the end of the run-in period and 2 weeks, 18 weeks and 42 weeks after treatment. (a) In the epithelium; (b) In the lamina propria

damage to the mucosa was seen after capsaicin treatment (thickness of the epithelium, thickness of basal membrane, number and size of glands).

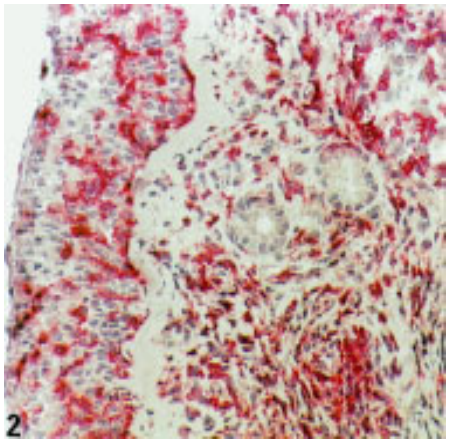
Inflammatory cells

The results are shown in Tables 2a and 2b. The mAb-ss staining showed red cells against a blue counter-stained background. T lymphocytes, small round cells, were abundantly present in the epithelium as well as the lamina propria (Fig. 2). Sometimes clusters of T cells were found in the epithelium or lamina propria (500-1000). The occurrence of these clusters did not differ between the groups. Langerhans cells, large dendritic cells, were found mostly in the

Table 2b.

Epithelium	Run in		2 weeks		18 weeks		42 weeks	
Treatment	*plac*	*caps*	*Plac*	caps	*Plac*	*caps*	*Plac*	*caps*
CD1	58	31	27	52	48	35	9	18
25%	51	15	18	16	21	17	9	18
75%	67	6	54	79	70	95	46	90
CD3	1372	591	616	1151	964	1262	856	1386
25%	1055	407	366	576	759	632	433	639
75%	3191	1237	1125	1457	1451	2172	2139	2552
CD25	50	36	47	82	24	43	28	45
25%	30	20	23	41	17	8	13	11
75%	93	75	79	141	51	97	54	83
CD68	384	312	267	477	320	573	380	544
25%	200	257	161	393	278	274	244	300
75%	911	675	752	571	831	984	524	965
BMK 13	23	11	16	51	18	15	16	39
25%	6	8	5	21	2	6	13	8
75%	45	43	53	89	52	77	38	93
Tryptase	199	192	194	302	212	205	171	248
25%	98	60	94	177	94	113	121	169
75%	281	402	494	448	387	395	213	479
Chymase	158	164	146	282	178	285	148	285
25%	100	93	62	112	123	119	98	189
75%	270	377	334	479	410	343	273	427
IgE	102	47	112	140	131	117	85	150
25%	55	30	45	75	34	36	35	55
75%	281	265	234	160	390	253	180	316

epithelium. Only a few were present in the lamina propria. Mast cells were found mostly in the lamina propria and hardly ever in the epithelium (Fig. 3). Eosinophils were hardly ever present in our material. Sometimes moderate infiltrates were found in the mucosa. The occurrence did not differ between the groups. The CD68 positive cells were large cells with a bright staining cytoplasm. This cell type was found to be equally distributed in both layers. No significant changes were found between treatment and placebo for any of the cells.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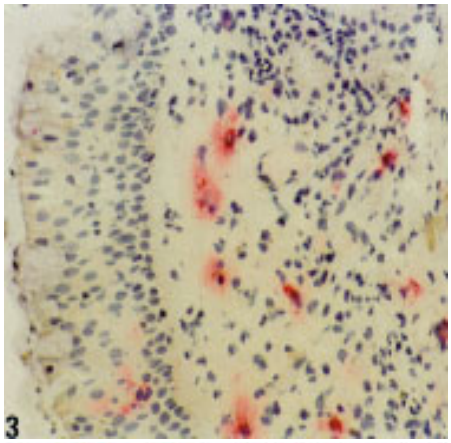
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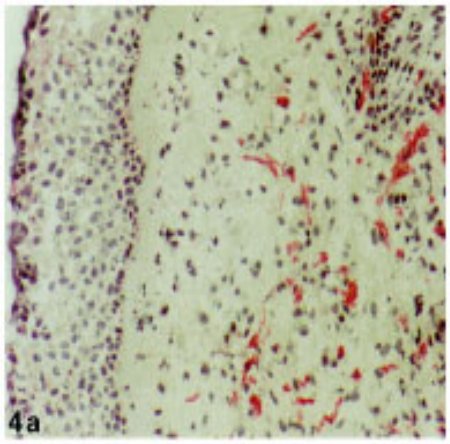
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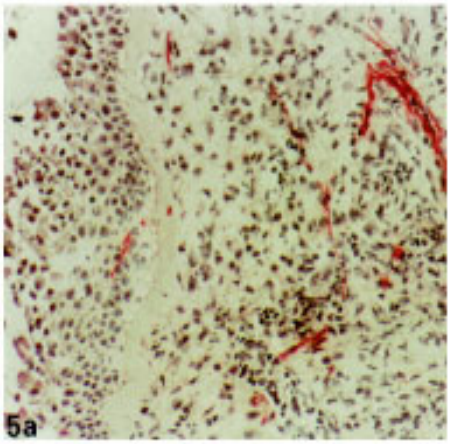
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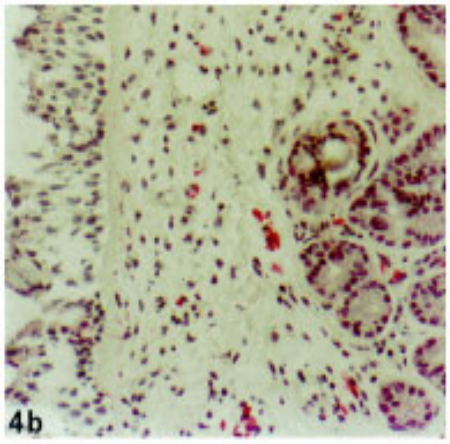
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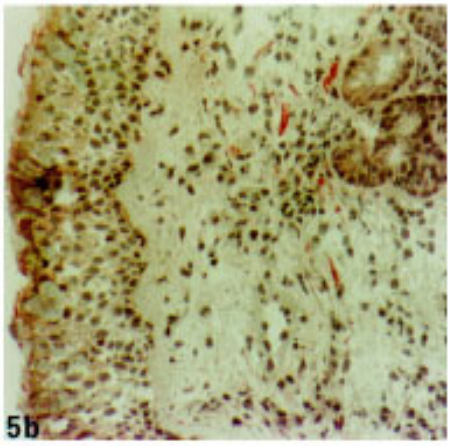
5b

Fig. 2. Lymphocytes in the nasal mucosa (CD3+). The epithelium, basal membrane and lamina propria can be distinguished. Positive cells stain red. Lymphocytes are abundantly present in both layers.

Fig. 3. Mast cells in the nasal mucosa (tryptase+). The different mucosal layers can be distinguished. Mast cells are not present in the epithelium, but are abundantly present in the lamina propria.

Fig. 4. Synaptophysine in the nasal mucosa. (a) Strong staining. Axial, transversal and longitidinal cut fibres can be distinguished. No signal is seen in the epithelium. (b) Weak staining. Mostly axial cut fibres are seen. One axial cut fibre is seen in the epithelium.

Fig. 5. Neurofilament in the nasal mucosa. (a) Strong staining. Mostly longitudinal cut fibres are seen in both the epithelium and lamina propria. (b) Weak staining. Some longitudinal cut fibres can be distinguished.

Neuronal staining

Synaptophysine and neurofilament staining showed red fibres cut at different angles (Figs 4 and 5). The rank correlation between any two observers varied from $r = 0.8$ to $r = 0.96$. The differences in ranking between any two observers varied between -8 and 11, with a mean and a median almost equal to zero, as expected. No significant differences between the two treatment groups were found for either synaptophysine or neurofilament staining.

Blood and urine

No significant changes were found for any of the blood or urine parameters.

DISCUSSION

The effect of capsaicin on nasal complaints and cellular mediators has already been described (1). To summarize, a 9 month amelioration of nasal complaints was seen without an effect on cellular mediators.

The mode of action of capsaicin is not clear, neither is the aetiology of idiopathic rhinitis. Proposed mechanisms for idiopathic rhinitis include the pos-

sibility of a chronic inflammatory disorder. The beneficial effect of capsaicin treatment could be the result of down-regulation of the inflammation, resulting in a reduction in the number of inflammatory cells. Knowledge on the effect of capsaicin provocation on nasal cellular homeostasis is limited to lavage studies following a single capsaicin dose. Philip et al. (10) described biphasic inflammatory-cell influx with neutrophils, eosinophils and mononuclear cells (in nasal lavage) up to 4 h following capsaicin provocation. Roche et al. (4) described an increase in neutrophils but not in other cells 10 min after capsaicin challenge. Whether or not this reflects a 'wash out' by increased nasal secretion, or an increase in mucociliary activity which could sweep cells out of the sinuses, or a transmigration of immunocompetent cells from the vessels through the nasal mucosa in the nasal lumen remains open for discussion (10). Our nasal biopsy study circumvents the 'wash out problem' since it allows study of all mucosal layers. In a pilot study with three patients (unpublished data) it was learnt that 2 weeks after the last capsaicin treatment a new steady state in nasal symptomatology was reached. If a correlation was to be found between the number of immunocompetent cells and nasal symptomatology, this would be the moment to ascertain it, it was hypothesized. The opportunity to study the direct effect of capsaicin on nasal immunocompetent cells ('provocation effect') was effectively missed since 2 weeks after cessation of the neurogenic stimulus the supposed capsaicin-induced neurogenic inflammation may have withered and would therefore not be detected. A biopsy taken directly after the first treatment was not considered opportunistic because six more treatments would follow.

No correlation was found between nasal symptomatology and any of the immunocompetent cells, for any of the biopsies. Nor were any significant cellular differences found between the placebo and the treatment group at any of the biopsy time points. This could mean that: (1) cells are not an integral part of the neurogenic response; or (2) the animal concept of neurogenic inflammation is not valid for the nasal airway, not even in inflamed airways when a neural hyperresponsiveness has developed (11).

The absence of correlation between cells and symptoms (which is congruent with our previous study in which a reduction in nasal immunocompetent cell numbers was found in a group of idiopathic rhinitic patients treated with fluticasone aqueous nasal spray without a reduction in nasal symptomatol-

ogy) (8), the absence of significant differences in the number of immunocompetent cells between capsaicin and placebo group, and the absence of an increase in cellular mediators (as a sign of cellular activation) following capsaicin challenge (1,12) raises the question of the relevance of the increase of immunocompetent cells in nasal lavage after capsaicin challenge.
Again it is concluded that immunocompetent cells are not involved in idiopathic rhinitis (7,8).
Reports on the effect of capsaicin treatment on neuronal tissue are not consistent. Lacroix et al. (13) showed a 50% decrease in CGRP-like immunoreactivity after capsaicin treatment of 16 patients with a drug-induced rhinitis suggesting depletion or atrophy of the unmyelinated sensory nerve fibres. In contrast, Wolf et al. (14), in a study of 123 patients, failed to show any reduction of peptidergic neurons within the nasal mucosa of 16 selected (uncharacterized) patients. He suggested a blockage of receptors as the mode of action. To quantify neuronal staining is difficult and often open to discussion, as nerve fibres may have a different diameter and can be cut at different angles, resulting in an abundant variation in staining morphology (Figs 4 and 5). A continuous ranking system was used. A very high Kappa for interobserver variability was found, suggesting a reliable quantification method. No significant differences were found between placebo and treatment groups for neurofilament or synaptophysine staining. These antibodies are pan-neurogenic markers. They do not allow discrimination between the adrenergic, the cholinergic and/or the peptidergic system. The data suggests that capsaicin does not induce gross changes in nervous tissue in the nasal mucosa in idiopathic rhinitis patients. Other signs of capsaicin-induced mucosal damage were not seen, and inflammatory cell densities were not affected. Possible changes in the peptidergic system (the supposed site of action of capsaicin) might not have been detected with these pan-neurogenic markers.
Capsaicin aqueous nasal spray does significantly improve nasal symptomatology in idiopathic rhinitic patients, without affecting cellular homeostasis or overall neurogenic staining up to 9 months after treatment.

REFERENCES

1. Blom HM, van Rijswijk JB, Garrelds IM et al. Intranasal capsaicin is efficacious in non-allergic non-infectious perennial rhinitis. A placebo controlled study. Clin Exp Allergy 1997; 27:796-801.
2. Joos GF, Germonpre PR, Pauwels RA. Neurogenic inflammation in human airways: is it important? [Editorial; Comment] Thorax 1995; 50(3):217-9.
3. Holzer P. Capsaicin; cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 1991 43(2):143-201.
4. Roche N, Lurie A, Authier S, Dusser DJ. Nasal response to capsaicin in patients with allergic rhinitis and in healthy volunteers: effect of colchicine. Am J Respir Crit Care Med 1995; 151(4):1151-8.
5. Wolf G. [New aspects in the pathogenesis and therapy of hyperreflexive rhinopathy]. [Rev] [German] Laryngol Rhinol Otol 1988; 67(9):438-45.
6. Wilde AD, Cook JA, Jones AS. The nasal response to isometric exercise in non-eosinophilic intrinsic rhinitis. Clin Otolaryngol 1996; 21(1):84-6.
7. Blom HM, Godthelp T, Fokkens WJ. et al. Mast cells, eosinophils and IgE-positive cells in the nasal mucosa of patients with visual analogue scaleomotor rhinitis. An immunohistochemical study. Eur Arch Otorhinolaryngol Suppl 1995; 1(9):S33-9.
8. Blom HM, Godthelp T, Fokkens WF et al. The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with a non-allergic non-infectious perennial rhinitis. J Allergy Clin Immunol 1997; 100:739-47.
9. Fokkens WJ, Vroom TM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. Rhinology 1988; 26(4):293-5.
10. Philip G, Sanico AM, Togias A. Inflammatory cellular influx follows capsaicin nasal challenge. Am J Respir Crit Care Med 1996; 153:1222-9.
11. Greiff L, Svensson C, Andersson M, Persson CG. Effects of topical capsaicin in seasonal allergic rhinitis [Comments]. Thorax 1995; 50(3):225-9.
12. Bascom R, Kagey-Sobotka A, Proud D. Effect of intranasal capsaicin on symptoms and mediator release. J Pharmacol Exp Ther 1991; 259(3):1323-7.
13. Lacroix JS, Buvelot JM, Polla BS, Lundberg JM. Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy 1991; 21(5):595-600.
14. Wolf G, Anderhuber W, Hauser-Kronberger C, Saria A. [Treatment of nonspecific hyper-reflectory rhinopathy (vasomotor rhinitis) with capsaicin]. [German] Laryngorhinootologie 1995; 74(5): 289-93.

CHAPTER VII

The direct effect of capsaicin aqueous spray on the nasal mucosa of idiopathic rhinitis patients: a double-blind placebo controlled biopsy study.

J.B. van Rijswijk, J.M. Keizer, E.L. Boeke E.J.J. de Groot, P.G.H. Mulder, H.M. Blom, R. Gerth van Wijk, and W.J. Fokkens

Submitted for publication

ABSTRACT

Background: To our knowledge, until now, data concerning the provocation effect of capsaicin on nasal cellular homeostasis in humans is limited to lavage studies following a single capsaicin application in allergic patients and healthy controls.
Objective: To get more insight in the pathophysiologic mechanism of idiopathic rhinitis and the direct mode of action of capsaicin on nasal mucosa cell counts and neurogenic staining.
Methods: We performed a double-blind placebo controlled nasal biopsy study in 30 strictly selected and well-defined idiopathic rhinitis (IR) patients challenged with either capsaicin or placebo. Biopsies were taken at baseline 2 weeks before provocation and 15 minutes and 1 hour after a single provocation with capsaicin or placebo. The cell densities of CD3, CD8, CD25, C-Kit, chymase, tryptase, BB1, IgE and BMK 13 and the neuronal staining with synaptophysine, neurofilament, VRL-1 were studied in both layers of the nasal mucosa.
Results: No significant difference in nasal mucosa cell counts and neurogenic staining were found 15 minutes and 1 hour after provocation. Only for CD3 in the epithelium 1 hour after provocation a significant higher cell count was found in the capsaicin group using the Mann-Whitney U test. Due to multiple testing this p-value of 0.011 for CD3 could have been easily caused by chance.
Conclusions: We conclude that capsaicin, after local anaesthesia of the nasal mucosa, does not affect cellular homeostasis or neurogenic staining 15 minutes and 1 hour after nasal provocation in this double-blind placebo controlled biopsy study. This strengthens us in our idea that inflammatory cells do not play a role in the mode of action of capsaicin and the aetiology of IR.

INTRODUCTION

Syndromes of chronic rhinitis with an unknown aetiology include nonallergic noninfectious perennial rhinitis, formerly referred to by us as NANIPER. In accordance with the ''World Health Organisation Initiative, Allergic Rhinitis and its Impact on Asthma''(1), we are using the term idiopathic rhinitis (IR) to describe this pathology from now on. IR, formerly also called vasomotor rhinitis, is a diagnosis of exclusion and is given to patients who suffer from perennial nasal congestion, rhinorrhea and/or sneezing with no identifiable aetiology. IR is unrelated to allergy, infection, structural lesions, polyposis and or other systemic diseases(2).

Patients with nonallergic rhinitis with eosinophilia syndrome (NARES) form another subgroup of nonallergic noninfectious perennial rhinitis patients. They have significant mucosal eosinophilia (a nasal smear with more than 25% eosinophils(3)) and respond well to nasal corticosteroids(4). In previous studies, we hardly found any patients with NARES in our IR patient groups, probably because we only selected IR patients in whom no therapeutic effect had been achieved with nasal corticosteroids(5-7).

The pathophysiology of IR is largely unknown. It is assumed that neurogenic mechanisms play an important role(8). Neuropeptides (CGRP, SP, etc.) are released from peptidergic neurons in the nasal mucosa after activation by unspecific stimuli, and can be responsible for the symptoms of IR(9-11).

Several studies have been published showing a therapeutic effect in IR patients for repeated topical applications of capsaicin(12-14). In recent papers, we showed that repeated administration of capsaicin in a double blind placebo-controlled trial led to a significant and long-term reduction of symptoms(15, 16).

Repeated application of capsaicin, the pungent agent in hot pepper, is known for its degeneration/desensitization effect on nonmyelinated peptidergic sensory C-fibres, possibly explaining its therapeutic effect(17, 18).

In a previous biopsy study we did not find any significant difference in nasal mucosa cell counts (T-lymphocytes, mast cells, IgE bearing cells, eosinophils and langerhans cells) between capsaicin and placebo treated patients 2 weeks, 3 months and 9 months after therapy although there was a significant therapeutic effect measured with visual analogue scale (VAS). Also no significant difference between both groups were found in pan-neurogenic staining of

nasal mucosa using neurofilament and synaptophysine(6).
To our knowledge, until now, data concerning the provocation effect of capsaicin on nasal cellular homeostasis in humans is limited to lavage studies following a single capsaicin application in allergic patients and healthy controls(19, 20).
To get more insight into the pathophysiologic mechanism of IR and the direct mode of action of capsaicin on nasal mucosa cell counts and neurogenic staining we performed a double-blind placebo controlled nasal biopsy study in 30 strictly selected and well-defined (Table 1) IR patients challenged with either capsaicin or placebo. Biopsies were taken 2 weeks before provocation at baseline (Biopsy I) and 15 minutes (biopsy II) and 1 hour (biopsy III) after a single provocation with capsaicin or placebo. The cell densities of T- lymphocytes(CD3, CD8, and CD25), mast cells(C-Kit, chymase, and tryptase), basophils (BB1), IgE bearing cells and eosinophils(BMK 13) and the neuronal staining with synaptophysine, neurofilament, and vaniloid receptor ligand – 1 (VRL-1), the 'capsaicin receptor'(21) were studied in both layers of the nasal mucosa.

MATERIALS AND METHODS

Subjects
Patients were admitted to the study if they had a history of nasal complaints such as nasal obstruction, sneezing and/or rhinorrhea for a period of over 1 year, which could not be attributed to allergic, nasal or paranasal infection, anatomical disorders affecting nasal function, pregnancy or lactation and/or systemic disorders (Table 1). They had to have used a nasal corticosteroid-spray for at least 6 weeks without any beneficial effect on their nasal symptoms. They were non-smokers not using medication affecting nasal function. All patients underwent nasendoscopy and patients with nasal polyps were excluded.
Patients with a diagnosis of IR scored their nasal complaints for a period of 2 weeks using a daily record chart (DRC) (Table 2). They were included in the study if periods of either clear nasal discharge, and/or sneezing and/or congestion persisted for an average of at least 1 hour a day for at least 5 days during a period of 14 days (22). Thirty patients participated under conditions

Table 1 Inclusion and exclusion criteria.

Inclusion criteria

Age between 16 and 65 years.
Negative Phadiatop (Pharmacia, Uppsala, Sweden)
Symptoms for more than 1 year.
Periods of nasal discharge, sneezing and congestion for an average of at least 1 hour per day for at least 5 days during a period of 14 days.
No beneficial effect of nasal corticosteroid spray (for a period of at least 6 weeks)

Exclusion criteria

Use of systemic or inhaled corticosteroids in the previous month.
Use of inhaled sodium cromoglycate or nedocromil sodium in the previous month.
Use of astemizole in the previous month.
Inability of the patient to stop taking medication affecting nasal function.
A serious and/or unstable disease.
Smoking (in the previous 6 months)
Nasal surgery in the previous 6 weeks.
Nasal polyps or a history of nasal polyps.
Significant anatomical abnormalities affecting nasal function.
Nasal or paranasal sinus infection (abnormal sinus X-ray).
Pregnancy or lactation

Table 2. Design of the daily record chart for defining nasal symptoms in IR patients.

Possible scores on the daily record chart	
Nasal blockage: (not being able to breathe freely through the nose) Clear nasal discharge: (runny nose)	0 = absent 1 = between 0-1h per half day 2 = between 1-2 h per half day 3 = more than 2 h per half day
Sneezing Coughing	0 = absent 1 = less than 5 periods per half day 2 = between 5-10 periods per half day 3 = more than 10 periods per half day
Green/Yellow mucus production:	0 = absent 1 = present

of informed consent (male/female: 14/16); mean age was 36 years (16 – 65 years). Procedures were approved by the local Medical Ethics Committee.

Study design

Patients were randomized in a double-blind placebo-controlled fashion 1:1 allocated either to provocation with placebo or capsaicin 2 weeks after a baseline biopsy (biopsy I) was taken. For this purpose a computer generated randomization list was prepared in blocks of 8 randomly permuted allocations. On the basis of this list the double-blind provocation medication(placebo or capsaicin) was prepared by the local pharmacist. 15 minutes (biopsy II) and 1 hour (biopsy III) after provocation a nasal mucosa biopsy was taken. The biopsy side for the baseline biopsy and the first biopsy 15 minutes after provocation were independently randomized. The second nasal mucosa biopsy 1 hour after provocation (biopsy III) was taken at the contralateral side of the first biopsy after provocation (biopsy II).

Provocation procedure

Provocation with capsaicin or placebo was preceded by three applications of xylometazoline-hydrochloride 0.1% (Otrivin^{R} nebulisator (1mg/ml Zyma, Breda, Holland)) in each nostril for decongestion. The nasal mucosa was then anaesthetized by three applications (10mg/puff) of lidocaine base (100mg/ml, Xylocaine^{R} 10% spray (Astra, Rijswijk, Holland)) in each nostril. To ensure good anaesthesia a pause of 15 minutes was introduced. The lips, columella and philtrum were covered with a petrolatum/ lanolin/ glycerin salve. The capsaicin solution (0.1 mmol/l) consisted of 30.3 mg pelargonic acid vanillylamide dissolved in 3 ml alcohol (96%) and diluted in 1 l NaCl solution (0.9%). As placebo, we used the capsaicin solvent only.

During provocation, 0.27 ml of solution (three applications) was sprayed into each nostril with a metered nasal spray (0.09 ml per actuation, coefficient of variation 4%).

Nasal biopsies

After randomization of the biopsy side (independently for biopsy I and II), specimen of nasal mucosa were taken from the lower edge of the inferior turbinate, about 2 cm posterior to the front edge, using a Fokkens forceps (Explorent, Tubingen, Germany), formally called Gerritsma forceps, with a cup diameter of 2.5 mm (23). Local anaesthesia was obtained by placing a cotton-wool carrier with 50 mg of cocaine and one drop of adrenaline (1:1000) under the inferior turbinate without touching the biopsy site. The specimen were embedded in Tissue-Tek II O.C.T. compound and frozen immediately.

Staining procedures

Monoclonal antibodies (mAb) directed against synaptophysine, neurofilament, CD3, CD8, CD25, C-Kit (CD 117), chymase, tryptase, BB1, IgE and BMK 13 (Table 3) were used together with the supersensitive

Table 3. Monoclonal antibodies used to study the muscosal biopsies.

Antibody	Specificity	Titre	Source
2F11	Neurofilament	1:50	Sanbio, Uden, NL
Sy38	Synaptophysine	1:25	Dakopatts, ITK, Uithoorn, NL
Rabbit anti-VRL-1	VRL-1	1:70	Chemicon, Temecula, Calif, USA
Leu4 Leu2 CD25 YB5.B8	CD3 CD8 IL2-r (CD25) CD 117	1:25 1:100 1:150 1:65	Becton Dickinson, Alphen aan de Rijn, NL
B7 G3	Chymase Tryptase	1:100 1:250	Chemicon, Temecula, Calif, USA
BB1	IgG1	1:150	A.F. Walls, South Hampton
MH25-1	IgE	1:250	Central laboratory of the Netherlands Red Cross Blood Transfusion service (CLB), Amsterdam, NL
BMK13	MBP	1:200	Sanbio, Uden, NL

immuno-alkaline phosphatase (ss-AP) method. Six micron thick serial sections of nasal mucosa were cut on a Reichert-Jung 2800e frigocut cryostat (Leica) and transferred to APES (amino-phosphate-ethylsilane) coated microscope slides (Starfrost), dried and stored at minus 70° C. When slides were used they were heated to room temperature, dried and fixed in ice cold acetone for 10 minutes at room temperature. They were then rinsed in phosphate-buffered saline (PBS, pH 7.8), placed in a semi-automatic stainer (Sequenza, Shandon), and incubated with normal goat serum (CLB, Amsterdam, the Netherlands) for 10 minutes. For blocking of endogenous avidine and biotin all antibodies were diluted in 1% blocking reagent in PBS (Roche 10961760). The sections were then incubated with the primary antibody for 60 minutes at room temperature. After this the sections were rinsed with PBS for 5 minutes and incubated for 30 minutes with a biotinylated goat anti-mouse (1:50) immunoglobuline antiserum (Biogenics, Klinipath, Duiven, the Netherlands), rinsed successively in PBS and incubated with streptavidin ss-AP (1:50) (Biogenics, Klinipath, Duiven, the Netherlands) for 30 minutes at roomtemperature. Slides were then rinsed again in PBS and TRIS buffer (0.2 mol/L, pH 8.5) and incubated for 30 minutes with new fuchsine (Chroma, Kongen, Germany) substrate (containing levamisole to block endogenous AP enzyme activity). Finally the sections were rinsed in distilled water, counterstained with Gill's hematoxylin and mounted in VectaMount (Vector, Burlingame, CA). Control staining was performed by substitution with PBS and incubation with an irrelevant mAb of the same subclass. Double staining with tryptase and chymase was also performed in an alkaline phosphatase procedure as previously described by our group (24). Staining with anti-VRL-1 was done using tyramide signal amplification (25).

Light-microscopic evaluation

Stained cells were counted in two sections of each biopsy specimen. The epithelium and lamina propria were evaluated separately. The total surface area of the sections and their main parts (i.e. the epithelium and the lamina propria) were estimated using the Leica Image Analysis System. The number of cells/mm^2 was calculated for the epithelium and the lamina propria.

The intensity, number and dimensions (width and length) of neuronal staining was semiquantified. Biopsies were ranked 1 to 30 by continuously comparing

the biopsies amongst another until all were ranked. In practice: a section was taken (at random), evaluated and put down. The next section was taken at random and graded for stronger or weaker staining compared with the previous section. At the end all sections were lined up.

Statistical analysis.
The Mann-Whitney U test was used to compare the distribution of the cell counts between the two provocation groups. The exact chi-square trend test was used to compare the ordinal score between the two provocation groups. A p-value < 0.05 was considered to indicate a significant difference.

RESULTS

The application of Xylocaine® 10% spray in the nasal airway was immediately followed by a painful sensation that was described by all subjects as most unpleasant. Patients did not complain of irritation of nose and lips during or after capsaicin/placebo application. We feel this study was effectively blinded for both patients and investigator.

Biopsy specimen
The sections of the nasal mucosa had an average surface area of 1.4 mm^2 and usually had a lining of ciliated columnar epithelium with or without goblet cells and/ or partially stratified cuboidal epithelium. The lamina propria usually consisted of a looser subepithelial cell-rich layer with most of the mucous glands and a deeper collagenous cell-poor layer. The sections were generally of good quality. All sections were sufficiently deep to assess both layers. No structural damage to the mucosa was seen after capsaicin provocation (thickness of the epithelium, thickness of basal membrane, number and size of glands) either after 15 minutes or 1 hour.
It was not possible to evaluate two biopsy specimens. One exclusion was made because the biopsy was to small and the other specimen was displaced.

Table 4a.
Median:

Epithelium	Biopsy I Run in		Biopsy II 15 min. after provocation		Biopsy III 1 h after provocation	
	Plac	Caps	Plac	Caps	Plac	Caps
CD3	198	170	155	215	104	248
25%	139	105	91	114	63	154
75%	337	216	254	371	167	287
CD8	118	142	192	118	128	121
25%	61	111	39	88	72	69
75%	236	204	241	257	180	307
CD25	13	10	12	16	14	17
25%	9	5	0	9	4	8
75%	36	51	25	35	31	38
C-Kit	13	9	23	14	19	8
25%	0	0	6	2	11	1
75%	39	31	45	52	29	29
Chymase	0	0	0	0	0	0
25%	0	0	0	0	0	0
75%	0	0	0	0	0	0
Tryptase	0	0	0	0	0	0
25%	0	0	0	0	0	0
75%	2	0	3	5	5	1
Try/Chym	3	1	3	5	0	4
25%	0	0	0	0	0	0
75%	21	8	36	14	26	13
BB1	0	0	0	0	0	0
25%	0	0	0	0	0	0
75%	0	0	0	0	0	0
IgE	102	27	42	128	37	41
25%	1	0	11	7	0	14
75%	160	148	206	191	167	80
BMK 13	5	0	7	8	5	8
25%	0	0	0	0	0	3
75%	22	13	35	22	18	29

Table 4a. and 4b.
Median cell numbers, 25th percentiles, and 75th percentiles for capsaicin (Caps) and placebo (Plac) provocation group of idiopathic rhinitis patients (both groups n=15) in epithelium (4a) and lamina propria (4b).

Table 4b.
Median:

Lamina propria	**Biopsy I Run in**		**Biopsy II 15 min. after provocation**		**Biopsy III 1 h after provocation**	
	Plac	Caps	Plac	Caps	Plac	Caps
CD3	345	404	294	291	298	370
25%	246	246	164	172	218	333
75%	444	644	466	695	393	428
CD8	125	177	121	169	130	169
25%	89	129	96	118	101	121
75%	224	272	161	258	180	213
CD25	21	33	31	45	24	47
25%	16	8	10	19	12	25
75%	28	57	39	89	54	67
C-Kit	84	32	88	87	57	46
25%	42	25	57	50	41	19
75%	93	59	129	129	93	77
Chymase	1	2	3	3	1	1
25%	0	0	0	0	0	0
75%	6	4	6	6	5	6
Tryptase	3	2	9	6	6	3
25%	1	0	2	1	3	1
75%	8	5	16	9	15	5
Try/Chym	125	96	135	135	127	115
25%	83	71	102	108	95	61
75%	149	118	207	185	170	157
BB1	0	1	1	1	1	1
25%	0	0	0	0	0	0
75%	2	3	4	3	3	5
IgE	109	64	105	144	107	90
25%	24	9	56	60	55	33
75%	143	105	235	187	211	127
BMK 13	17	10	30	18	23	46
25%	6	5	11	5	6	4
75%	42	20	72	39	44	83

Inflammatory cells

The results are shown in tables 4a en 4b. The mAB-ss-AP staining showed red cells against a blue counterstained background. T-lymphocytes, small round cells, were abundantly present in the epithelium as well as in the lamina propria. Sometimes clusters of T-cells were found in the epithelium or lamina propria. The occurrence of these clusters did not differ between the groups. Mast cells and basophils were found mostly in the lamina propria and hardly

ever in the epithelium. Eosinophils were hardly ever present in this material. Sometimes moderate infiltrates were found in the lamina propria. Again the occurrence did not differ between the groups.
Only for CD3 in the epithelium 1 hour after provocation a significant higher cell count was found in the capsaicin group using the Mann-Whitney U test. Due to multiple testing the p-value of 0.011 for CD3 could have been easily caused by chance.

Neuronal staining
The staining for synaptophysine, neurofilament and VRL-1 showed red fibres cut at different angles. For VRL, synaptophysine and neurofilament no significant differences were found between the two provocation groups using the exact chi-square trend test.

DISCUSSION

The therapeutic effect of capsaicin in strictly selected IR patients has repeatedly been demonstrated by our group and others (12-16) and has been introduced in our daily clinical practice. Unfortunately the mode of action of capsaicin and the aetiology of IR still remains uncertain.
Nasal capsaicin provocation results in rhinorrhea, nasal blockage and sneezing (26). This sensory neural stimulation may produce these effects either through a orthodromic, central neural reflex, associated with efferent parasympathetic neurotransmission, and or via anti-dromic release of neuropeptides from sensory neurons (27). Repeated applications of capsaicin, however, lead to desensitization and even degeneration of peptidergic unmyelinated sensory C-fibres(18, 28).
Therefore the hypothesis, suggested by Wolf, that an hyperactive non-adrenergic non-cholinergic peptidergic neuronal system is the underlying pathophysiology in IR may offer an explanation for the beneficial effect of capsaicin with these patients.
This hypothesis was corroborated by Lacroix, who reported an increased concentration of neuropeptides in a group of chronic IR patients(11), improvement of symptoms by local treatment of capsaicin giving a 50% reduc-

tion in CGRP-Li content in nasal biopsies (17), and a correlation between symptom intensity and CGRP-Li concentration in nasal mucosa (29).
In our previous work we did not find any significant difference between mucosal inflammatory cellular infiltrates of symptomatic IR patients and healthy controls (5, 7). This in contrast with allergic rhinitis where mucosal inflammatory cellular infiltrates are correlated with nasal complaints (30, 31). In two other studies, we failed to ascertain a relation between the number of immunocompetent cells and nasal complaints in IR patients. A significant reduction of immunocompetent cells in the nasal mucosa of IR patients treated with nasal steroids (fluticasone aqueous nasal spray) was not accompanied by a reduction in nasal complaints(32) and, inversely, a significant reduction in nasal complaints in a group of IR patients treated with topical capsaicin aqueous nasal spray was not accompanied by a change in inflammatory mediators (15) or a reduction/change in the numbers of inflammatory cells in nasal mucosa biopsies 2 weeks, 3 months, and 9 months after the treatment period (6).
In the present study we did not find any significant difference in nasal mucosa cell counts and neurogenic staining 15 minutes and 1 hour after provocation. The difference found for CD3 in the epithelium 1 hour after provocation is interpreted by us as caused by chance (multiple testing). This strengthens us in our idea that inflammatory cells do not play a role in the mode of action of capsaicin and the aetiology of IR.
It is therefore not surprising, that our findings are in contrast with the results of nasal challenge with capsaicin in subjects with allergic rhinitis. Philip described biphasic inflammatory-cell influx with neutrophils, eosinophils and mononuclear cells in nasal lavage upto 4 hours following capsaicin provocation in ten patients with allergic rhinitis and in 10 healthy controls (20). Roche described an increase in neutrophils 10 minutes after capsaicin challenge in eight patients with allergic rhinitis but not in eight healthy controls (19). Whether or not this reflects a ‘wash out’ by increased nasal secretion, an increase in mucociliary activity which could sweep cells out of the sinuses, or a transmigration of immunocompetent cells from the vessels through the nasal mucosa in the nasal lumen remains open for discussion (20).
Neither Roche nor Philip anaesthetized the nasal mucosa prior to the capsaicin administration.

The inflammatory-cell influx they found after capsaicin provocation, as opposed to our results, might well be caused by a protective orthodromic, central neural reflex due to the nociceptive stimulation (pain) of capsaicin in combination with allergic nasal inflammation. In our model this reflex will be blocked by the local anaesthesia applied prior to capsaicin provocation, possibly preventing this protective inflammatory-cell influx. Whether this reflex and or an anti-dromic release of neuropeptides from sensory neurons was the underlying mechanism for their post capsaicin inflammatory-cell influx remains open for debat, the more so as Roche, as opposed to Philip, did not found this inflammatory-cell influx in the healthy control group.
These and previous findings, however, do not discard Wolfs hypothesis of a hyperactive non-adrenergic non-cholinergic peptidergic system, as the activity of this system was not measured. A functional hyperactivity of this system, not captured by histological changes, could still be the underlying pathophysiological process in IR also providing an explanation for the nasal hyperreactivity in IR demonstrated with cold dry air provocation (33) and the significant reduction in hyperreactivity up to 9 months after capsaicin therapy (16).
Another possibility, raised by Sanico, is a hyper- or dysaesthesia at the central nervous system (CNS) level as an explanation for IR (8). This would explain why we do not find any significant differences in cell counts and neurogenic staining between capsaicin and placebo provocation in this study and between IR patients and normals. According to this theory a functional or numerical downregulation of the nonmyelinated peptidergic sensory C-fibres would explain the therapeutic effect of intranasal capsaicin application (8).
To quantify neuronal staining is difficult as nerve fibres may have a different diameter and can be cut at different angels, resulting in an abundant, “spaghetti like”, variation in staining morphology. Therefore (small) alterations in the peptidergic neuronal system might not have been detected in this study.
We cannot exclude the possibility that we overlooked the presence of other allergies, not assessed in our patients. Moreover, it has been hypothesised that IR is based on a local, occult allergy (34-36). The diagnosis of IR is made by exclusion. An allergy test is not 100% sensitive and absence of systemic manifestations of atopic disease, such as a positive skin prick test

or RAST, may not reflect the processes in the nose - a small shock organ. However, in seasonal or perennial allergic rhinitis, increased numbers of inflammatory cells, such as Langerhans cells, IgE positive cells, basophils and eosinophils, can be found in the nasal mucosa as a sign of inflammation (25, 30, 37). In this strictly selected group of IR patients we did not find any of the histological characteristics of allergic rhinitis or NARES (Table 4a and 4b). We therefore discard local allergy or NARES as being the underlying pathophysiology for IR in this group of patients.
We conclude that capsaicin, after local anaesthesia of the nasal mucosa, does not affect cellular homeostasis or neurogenic staining 15 minutes and 1 hour after nasal provocation in this double-blind placebo controlled biopsy study. This strengthens us in our idea that inflammatory cells do not play a role in the mode of action of capsaicin and the aetiology of IR.

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REFERENCES

1. Bousquet C. World Health Organization Initiative Allergic Rhinitis and its Impact on Asthma (ARIA). Geneva: World Health Organisation; 2001.
2. Philip G, Togias AG. Nonallergic rhinitis. Pathophysiology and models for study. Eur Arch Otorhinolaryngol Suppl 1995;1(3):S27-32.
3. Mikaelian AJ. Vasomotor rhinitis. [Review]. Ear Nose Throat J 1989;68(3):207-10.
4. Small P, Black M, Frenkiel S. Effects of treatment with beclomethasone dipropionate in subpopulations of perennial rhinitis patients. J Allergy Clin Immunol 1982;70(3):178-82.
5. Blom HM, Godthelp T, Fokkens WJ, Kleinjan A, Holm AF, Vroom TM, et al. Mast cells, eosinophils and IgE-positive cells in the nasal mucosa of patients with vasomotor rhinitis. An immunohistochemical study. Eur Arch Otorhinolaryngol Suppl 1995;1(9):S33-9.
6. Blom HM, Severijnen LA, van Rijswijk JB, Mulder PG, Gerth van Wijk R, Fokkens WJ. The long-term effects of capsaicin aqueous spray on the nasal mucosa. Clin Exp Allergy 1998;28 (11):1351-8.

7. Van Rijswijk JB, Blom HM, Kleinjan A, Mulder PGH, Rijntjes E, Fokkens WJ. Inflammatory cells seem not to be involved in idiopathic rhinitis. Rhinology 2003;41:25-30.
8. Sanico A, Togias A. Noninfectious, nonallergic rhinitis (NINAR): considerations on possible mechanisms. Am J Rhinol 1998;12(1):65-72.
9. Baraniuk JN, Kaliner MA. Neuropeptides and nasal secretion. J Allergy Clin Immunol 1990;86(4 Pt 2):620-7.
10. Fang SY, Shen CL. Neuropeptide innervation and neuroendocrine cells in allergic rhinitis and chronic hypertrophic rhinitis. Clin Exp Allergy 1998;28(2):228-32.
11. Lacroix JS, Kurt AM, Pochon N, Bretton C, Lunberg JM, Deshusses J. Neutral endopeptidase activity and concentration of sensory neuropeptide in the human nasal mucosa. Eur Arch Otorhinolaryngol 1995;252:465-468.
12. Wolf G, Anderhuber W, Hauser-Kronberger C, Saria A. [Treatment of nonspecific hyper-reflectory rhinopathy (vasomotor rhinitis) with capsaicin]. Laryngorhinootologie 1995;74(5):289-93.
13. Riechelmann H, Davris S, Bader D. [Treatment of perennial non-allergic rhinopathy with capsaicin]. Hno 1993;41(10):475-9.
14. Marabini S, Ciabatti PG, Polli G, Fusco BM, Geppetti P. Beneficial effects of intranasal applications of capsaicin in patients with vasomotor rhinitis. Eur Arch Otorhinolaryngol 1991;248(4):191-4.
15. Blom HM, van Rijswijk JB, Garrelds IM, Mulder PG, Timmermans T, Gerth van Wijk R. Intranasal capsaicin is efficacious in non-allergic, non-infectious perennial rhinitis. A placebo-controlled study. Clin Exp Allergy 1997;27(7):796-801.
16. Van Rijswijk JB, Boeke EL, Keizer JM, Mulder PGH, Blom HM, Fokkens WJ. Intranasal capsaicin reduces nasal hyperreactivity in idiopathic rhinitis: a double-blind randomised application regimen study. Allergy 2003;in press.
17. Lacroix JS, Buvelot JM, Polla BS, Lundberg JM. Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy 1991;21(5):595-600.
18. Kitajiri M, Kubo N, Ikeda H, Sato K, Kumazawa T. Effects of topical capsaicin on autonomic nerves in experimentally-induced nasal hypersensitivity. An immunocytochemical study. Acta Otolaryngol Suppl 1993;500(2):88-91.
19. Roche N, Lurie A, Authier S, Dusser DJ. Nasal response to capsaicin in patients with allergic rhinitis and in healthy volunteers: effect of colchicine. Am J Respir Crit Care Med 1995;151(4):1151-8.
20. Philip G, Sanico AM, Togias A. Inflammatory cellular influx follows capsaicin nasal challenge. Am J Respir Crit Care Med 1996;153(4 Pt 1):1222-9.
21. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D.

The capsaicin receptor: a heat-activated ion channel in the pain pathway [see comments]. Nature 1997;389(6653):816-24.
22. Mygind N, Weeke B. Allergic and vasomotor rhinitis: Clinical aspects. Copenhagen: Munksgaard; 1985.
23. Fokkens WJ, Vroom TM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. Rhinology 1988;26(4):293-5.
24. Kleinjan A, Godthelp T, Blom HM, Fokkens WJ. Fixation with Carnoy's fluid reduces the number of chymase positive mast cells. Not all chymase positive mast cells are also positive for tryptase. Allergy 1996;51(9):614-20.
25. Braunstahl GJ, Overbeek SE, Fokkens WJ, Kleinjan A, McEuen AR, Walls AF, et al. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. Am J Respir Crit Care Med 2001;164(5):858-65.
26. Baraniuk JN. Sensory, parasympathetic, and sympathetic neural influences in the nasal mucosa. J Allergy Clin Immunol 1992;90(6 Pt 2):1045-50.
27. Lundblad L, Saria A, Lundberg JM, Anggard A. Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons. Acta Otolaryngol 1983;96(5-6):479-84.
28. Holzer P. Capsaicin;cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 1991;43(2):143-201.
29. Lacroix JS, Pochon N, Lundberg JM. Increased concentration of sensory neuropeptide in the nasal mucosa from patients with non-allergic chronic rhinitis. In: The New frontiers of otorhinolaryngology in europe; 1992; Sorrento, Italy: Monduzzi Editore S.p.A., Italy; 1992. p. 59-63.
30. Fokkens WJ, Holm AF, Rijntjes E, Mulder PG, Vroom TM. Characterization and quantification of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. Int Arch Allergy Appl Immunol 1990;93(1):66-72.
31. Godthelp T, Holm AF, Fokkens WJ, Doornenbal P, Mulder PG, Hoefsmit EC, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;97(3):800-11.
32. Blom HM, Godthelp T, Fokkens WF, Kleinjan A, Mulder PGM, Rijntjes E. The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with a non-allergic non-infectious perennial rhinitis. J Allergy Clin Immunol 1997;100(..):739-47.
33. Braat JP, Mulder PG, Fokkens WJ, Gerth van Wijk R, Rijntjes E. Intranasal cold dry air is superior to histamine challenge in determining the presence and

degree of nasal hyperreactivity in nonallergic noninfectious perennial rhinitis. Am J Respir Crit Care Med 1998;157(6 Pt 1):1748-55.
34. Carney AS, Hooi D, Powe DG, Huskisson RS, Jones NS. Autoanti-IgE antibodies in patients with allergic and idiopathic rhinitis. Clin Otolaryngol 2001;26(4):298-301.
35. Powe DG, Huskisson RS, Carney AS, Jenkins D, Jones NS. Evidence for an inflammatory pathophysiology in idiopathic rhinitis. Clin Exp Allergy 2001;31(6):864-72.
36. Shatkin JS, Delsupehe KG, Thisted RA, Corey JP. Mucosal allergy in the absence of systemic allergy in nasal polyposis and rhinitis: a meta-analysis. Otolaryngol Head Neck Surg 1994;111(5):553-6.
37. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. J Allergy Clin Immunol 1992;89(4):877-83.

CHAPTER VIII

General discussion and conclusions

INTRODUCTION

Although our knowledge about nonallergic noninfectious rhinitis and its possible cause has increased the last decades, still about 50% of the patients with nonallergic noninfectious rhinitis has to be classified as suffering from idiopathic rhinitis, or rather e causa ignota. This, combined with the limited and frequently insufficient treatment options available, remains a frustrating burden for almost every clinically active ENT specialist and allergologist, let alone all idiopathic rhinitis (IR) patients, often seeking second and third opinions because of continuing complaints despite up to date treatment. The care for the IR patients and the above-mentioned frustration with the often disappointing results of treatments available were the guidelines for the research described in this thesis.

EVOLUTION IN NOMENCLATURE

The publication of the the "World Health Organisation Initiative, Allergic Rhinitis and its Impact on Asthma" (ARIA) in 2001 brought more clarity about the nomenclature of nonallergic rhinitis (1). In accordance with this publication the term idiopathic rhinitis (IR) is now commenly used in the world literature to describe syndromes of chronic rhinitis with an unknown aetiology. Before this important initiative almost every research group used its own description. For example IR was formerly referred to by us as NANIPER (2), where nowadays NANIPER is used as a collective term to describe all, known and unknown, nonallergic noninfectious perennial rhinitis syndromes, among others encompassing IR. Other terms, like NINAR (noninfectious, nonallergic rhinitis) (3), intrinsic rhinitis (4), hypertrophic rhinitis (5) and vasomotor rhinitis (6), are also purely descriptive and should not longer be used to prevent misunderstandings. Therefor, and for the sake of clarity and uniformity, the term NANIPER, used in the original articles forming Chapter III and VI, was replaced with idiopathic rhinitis.

DIAGNOSTIC CRITERIA FOR IDIOPATHIC RHINITIS

To exclude all known prevailing causes of chronic rhinitis one should at least take a proper history (medication, smoking in previous 6 months, occupation, etc.), adequately exclude commonly occurring inhalation allergies (skin prick test and or specific serum IgE measurement) and perform rhinoscopia anterior and nasendoscopy to exclude gross anatomical aberrations and nasal polyps.
The mucosa of the nose and sinus are contiguous and thus chronic nasal complaints can also be induced by a (accompanying) chronic sinusitis. When in doubt of a possible chronic sinusitis one should not hesitate to perform CT-scan imaging. However, if the history and the nasendoscopy lack criteria pointing at possible sinus problems, CT-scan imaging is not obligatory for diagnosing IR.

Nasal complaints as a IR selection-criterion
After having excluded all known causes of chronic rhinitis one is left with a group of patients with nasal complaints of unknown pathology (IR). This means that the studied patient group is probably a melting pot of patients suffering from nasal complaints, with presumably variable pathogenesis. To study, select and define a group of patients, and more, measure the effects of interventions, positive criteria are needed to make the group as homogeneous as possible. As IR is solely diagnosed on patients complaints we use (and have used in all our previous studies to IR) a daily record chart on which patients have to reach a minimum symptom score to be classified as IR patient. The minimum is set using as a basis the definition of rhinitis put forward by Mygind and Weeke (8). In affected patients, periods of nasal discharge, sneezing and / or congestion have to persist for an average of at least 1 hour per day on at least five days during a period of fourteen days.

FACTS AND HYPOTHESES ON IDIOPATHIC RHINITIS, HOW DO THE DATA FROM THIS THESIS FIT WITHIN

Pathophysiology:

Chronic inflammation

One of the proposed pathophysiological mechanisms for IR includes a chronic inflammatory disorder of antigenic (local allergy) or neurogenic nature (9-11). A pivotal characteristic in the pathophysiological concept of inflammation is an influx of inflammatory cells in the affected tissue. Knani reported increased mediator levels in IR suggesting an involvement of inflammatory cells (12). Mast cells were implicated by Abe and Terrahe (13, 14). However, Braunstein and Hua failed to find any evidence for neurogenically induced mast cell degranulation. Despite the fact that substance P can induce histamine release from isolated human skin mast cells in vitro, it does not so in the human nasal mucosa or in rat trachea in vivo (15, 16). Also the generally sub-optimal result of antihistamine therapy in IR patients tells against an important role of histamine in the pathophysiology of IR (17).

In symptomatic allergic rhinitis patients, an increase of inflammatory cells has been observed in the nasal mucosa and this increase is positively correlated to nasal complaints (18-20).

In chapter V we did not found any significant difference for nasal mucosal lymphocytes, antigen-presenting cells, eosinophils, macrophages, monocytes, mast cells and other IgE-positive cells between IR patients and controls.

In chapter III and VI, we failed to ascertain a relation between the number of immunocompetent cells and nasal complaints in IR patients. A significant reduction in nasal complaints in a group of IR patients treated with topical capsaicin aqueous nasal spray was not accompanied by a change in inflammatory mediators or a reduction in the numbers of inflammatory cells. Furthermore, Blom found a significant reduction of immunocompetent cells in the nasal mucosa of IR patients treated with nasal steroids (fluticasone aqueous nasal spray) that was not accompanied by a reduction in nasal complaints (7).

In chapter VII no significant differences in nasal mucosa cell counts, between the capsaicin and placebo provocated group, were found 15 minutes and 1 hour after intranasal provocation in a double-blind placebo controlled biopsy study.

Considering the aforementioned we conclude that inflammatory cells are not involved in our meticulously selected, non smoking, IR patient groups and that a chronic inflammatory disorder (e.g. local allergy) does not seem to be one of the important pathophysiological mechanisms in IR. We also conclude that NARES patients were not included in our IR patient groups because eosinophils are not found in the mucosa of our patients.

The NANC-hypothesis

One of the prevailing hypotheses nowadays concerning the pathophysiology of IR is the one, among others suggested by Wolf (21), holding a hyperactive nonadrenergic noncholinergic (NANC) peptidergic neural system responsible for the pathophysiology in IR (further summarised as the NANC-hypothesis). One of the firsts to suggest the NANC-hypothesis in IR was Lundblad in 1983, extrapolating study results on rat nasal mucosa with substance P and capsaicin (22).

The NANC-hypothesis was corroborated by Lacroix, who reported an increased concentration of neuropeptides in a group of chronic IR patients (23), improvement of symptoms by local treatment of capsaicin giving a 50% reduction in CGRP-Li content in nasal biopsies (24), and a correlation between symptom intensity and CGRP-Li concentration in the nasal mucosa (25).

Perivascular and intra-epithelial NANC-sensory nerve fibres contain neuropeptides (including VIP, substance P (SP), calcitonin gene related peptide (CGRP), id.) which are demonstrated in the nasal mucosa of various mammals including man (26, 27). The actions of these neuropeptides are limited by neutral endopeptidase degradation (28). These neuropeptides are locally released from peptidergic neurons (antidromic release), mainly unmyelinated sensory C-fibres, in the nasal mucosa after activation by unspecific stimuli, and can be responsible for the symptoms of IR (5, 23, 29). Stimulation can be induced by inflammatory mediators, like histamine and bradykinin but also by a number of inhaled irritants like nicotine, cigarette smoke, formaldehyde and capsaicin (30-32).

With the exemption of Lacroix's study, reporting an increased concentration of neuropeptides in a group of chronic IR patients (23), solid proof that the NANC-hypothesis forms one of the underlying pathophysiologic mechanisms

in IR in human beings is still lacking. The fact that intranasal capsaicin is an effective therapy in the majority of IR patients, reported by several authors in the last decade (33-35), is till now probably the hardest indication for the role of the NANC-hypothesis in IR.
In our histology studies we did not find any significant difference in pan-neurogenic staining of nasal mucosa using neurofilament and synaptophysine between capsaicin and placebo treated patients 2 weeks, 3 months and 9 months after therapy (chapter VI) although there was a significant therapeutic effect measured with visual analogue scale (chapter 3). Also Wolf was unable to show a reduction of NANC-fibres in the nasal mucosa of IR patients after successful capsaicin treatment (33) using monoclonal antibodies directed against SP, VIP and polyclonal antibodies against CGRP and NPY.
Fang reported on neuropeptide tissue concentrations and neuroendocrine cell densities in normals and IR patients (5). No significant differences were found. Unfortunately, in spite of elegant neuropeptide quantification methods, patients were simple characterized as suffering from chronic hypertrophic rhinitis without mentioning of any in- or exclusion criteria.
In chapter VII no significant differences were found between the capsaicin and placebo provocated group in nasal mucosa neurogenic staining using monoclonal antibodies directed against synaptophysine, neurofilament and VRL-1, 15 minutes and 1 hour after intranasal provocation.
With the exception of VRL-1, we used pan-neurogenic markers, not allowing discrimination between the adrenergic, cholinergic and or the peptidergic system. Also quantifying neuronal staining is difficult as nerve fibres may have a different diameter and can be cut at different angels, resulting in an abundant, 'spaghetti like', variation in staining morphology. Therefore small alterations in the peptidergic neuronal system might have been missed.
The main problem with using histology in studying the role of the NANC-hypothesis in IR is that one is only looking for inferential evidence, as the activity of the system itself is not measured. Therefore our lack of positive histology criteria regarding the NANC-hypothesis in IR may not let us conclude that this hypothesis does not play an important role in IR. It might well be that capsaicin exerts its effect of sensory neuropeptide depletion by receptor modulation instead of neurotoxity. Wolf suggested capsaicin receptor blockage as a possible explanation for the capsaicin treatment effect (33). Although sounding attractive it seems improbable that capsaicin receptor blockage

alone can result in the long lasting therapeutic effect for capsaicin observed in IR patients.
Recapitulating, and keeping in mind the beneficial action of intranasal capsaicin in IR, one can state that the NANC-hypothesis probably forms one of the important pathophysiological mechanisms underlying IR, although decisive data is still missing.

Hyper- or dysesthesia at the CNS level
Another possibility, raised by Sanico, is a hyper- or dysesthesia at the central nervous system (CNS) level as an explanation for IR (3). An abnormally increased nasal perceptual acuity as the underlying pathophysiology in IR would explain the lack of changes/differences in cell counts and neurogenic staining in the several studies mentioned above. According to this theory a functional or numerical downregulation of the unmyelinated peptidergic sensory C-fibres would also explain the therapeutic effect of intranasal capsaicin application, reducing perceptive signals to the sensory cortex (3). One might speculate that the central 'nasal perception gauge' is re-adjusted by a vicious circle of environmental irritants and changes in atmospheric conditions, perceived as an ever irritating stimulans at the CNS level, giving rise to the protective responses hereupon like rhinorrhea, vascular congestion and sneezing.
Sounding attractive this "hyperesthesia at the CNS level" probably plays a role in the IR pathology, as CNS neuronal plasticity may play a role in other chronic diseases like headache and low backpain (36, 37). Neuronal hyperexcitability, which apparently is a key phenomenon in many types of chronic pain, can result in changes in the nervous system from the level of the peripheral nociceptor to the highest cortical centers in the brain (38).
Whether it really forms a solitary subtype of IR will be very difficult to prove due to the key role allocated to the central nervous system (CNS) in this theory. Elaborating on this theme one might consider treating IR patients with CNS directed medication like antidepressants or other neuro-modulating agents trying to achieve a resetting of the central 'nasal perception gauge'.

Pollutional and meteorological factors:

Epidemiological studies traditionally focused on the relationship of meteorolgical conditions, pollution and pollen with symptoms of bronchial hyperreactivity, asthma and allergy (39-41). In a recent article Braat et al focused on the relation between complaints in IR patients and meteorological factors(42). He showed that minor pollution and meteorological disturbances can result in substantial changes in nasal reactivity symptoms in IR patients but not in controls. An increasing number of in- and outdoor pollutants is recognised as being irritative for the nasal mucosa. Among these are dust, ozone, black smoke, sulfur dioxide, volatile organic compounds, formaldehyde, and environmental tobacco smoke (43-45). Whether there is a relationship between frequent/longlasting exposure to these agents and the development of IR is unknown although there is some circumstantial experimental evidence of impaired nasal function after exposure in laboratory settings (46-48). Whether the increased nasal hyperreactivity in IR patients to these agents is post or propter remains open for further investigations.

Nasal Hyperreactivity:

Nasal hyperreactivity to non-specific stimuli is a common and characteristic feature of patients with chronic rhinitis. Hyperreactivity only describes the increased reactivity of the nasal mucosa to 'nonspecific'stimuli such as smoke, strong odors and other irritants but does not point to any cause of the disease. In addition, patients with allergic rhinitis usually complain of hyperreactivity to non-allergic stimuli, obviously as a direct result of allergic inflammation (49). Several studies showed a positive correlation between nasal hyperreactivity and airway inflammation in allergic rhinitis (50-52). Patients with ongoing allergic rhinitis seem to be in a continuous late phase state of eosinophilia and increased mediator release, a condition that can explain priming and nonspecific hyperreactivity of the nasal mucous membrane (53, 54).

With his publication on CDA provocation in 1998 (55), our dear and recently too young deceased colleague dr. Braat provided us with a grateful used (Chapter IV), diagnostic tool for measuring hyperreactivity and monitoring treatment effect in IR.

In chapter IV we found, along with a significant decrease in visual analogue

scores for nasal complaints, a significant reduction in cold dry air dose responsiveness up to 9 months after start of intranasal capsaicin treatment, reflecting a decrease in nasal hyperreactivity. Taking into account the accompanying absence of a significant change in peak nasal inspiratory flow (PNIF) and acoustic rhinometry, and the fact that the values of PNIF and acoustic rhinometry did not differ from values found in normal controls, it is our opinion that one of the most important characteristics that results in symptoms in IR patients is increased hyperreactivity of the nasal mucosa rather than decreased patency.

This seems to be in agreement with the observation of a significant increase in nasal airway resistance after 3 repeated forced expirations through the nose in IR patients but not in healthy controls (56). Likewise, no significant differences were found between baseline nasal airway resistance and peak nasal expiratory flow rate measurements in IR patients and healthy controls in this study.

CDA challenge is a natural stimulus, although exposure conditions are artificial. CDA is thought to provoke neurogenic reflex mechanisms in the nasal mucosa (31, 57). This, combined with the significant reduction in cold dry air dose responsiveness up to 9 months after start of intranasal capsaicin treatment found in chapter IV, also points in the direction of the NANC hypothesis, that a hyperactive nonadrenergic noncholinergic peptidergic neural system is the underlying pathophysiology in IR.

Capsaicin desensitisation in patients with perennial allergic rhinitis to house dust mite (HDM) with a comparable treatment protocol as was used in the study of chapter III was lacking therapeutic effect in a placebo controlled trial (72). Probably the nasal hyperreactivity induced by an allergic inflammation, such as HDM allergic rhinitis, is mainly dominated by inflammatory events (a continuous late phase state of eosinophilia and increased mediator release) and thereby not modified by interference at the level of C-fibers.

CAPSAICIN THERAPY AND ITS SAFETY IN IDIOPATHIC RHINITIS

The unmyelinated sensory C-fibres or 'pain receptors' are specifically sensitive to capsaicin (8-methyl-N-vanillyl-6-nonenamide), the pungent agent of hot red pepper (58, 59). Nasal capsaicin provocation results in rhinorrhea, nasal blockage and sneezing(60). This sensory neural stimulation may produce these effects either through an orthodromic, central neural reflex, associated with efferent, predominantly parasympathetic, neurotransmission, and or via an antidromic, afferent, local release of neuropeptides from sensory neurons (22). Repeated applications of capsaicin lead to desensitisation and even degeneration of peptidergic unmyelinated sensory C-fibres (61, 62) possibly explaining its therapeutic effect in IR. The efficiency of capsaicin in IR has been reported on by many authors (33-35) but was still not generally accepted, and much less applied, as one of the treatment options in IR. In chapter III and IV we showed in a double-blind placebo controlled fashion that intranasal capsaicin application ameliorates nasal symptoms for at least 9 months. It is our opinion that with these studies objective and hard data has been provided concerning the favourable effect of intranasal capsaicin in IR. However, direct observations explaining the efficacy and working mechanisms of capsaicin are still lacking, comparable and in parallel with the lack of knowledge about the pathophysiology of IR. Although classical observations on functional desensitisation of nociceptors by capsaicin may explain its beneficial effects, the recent discovery of a range of receptors which respond to capsaicin, menthol, and temperature and their expression in subsets of sensory nerve fibres, provides an exciting prospect towards advancing our understanding and treatment of IR and the more precise modes of action of capsaicin (63-65).

In chapter IV no significant changes in safety data (smell, blood pressure, heart rate) were found during and after a treatment period of 10 days with intranasal capsaicin once every second or third day for a total of five applications. This observation, combined with the fact that up to the writing of this discussion we never had any serious and or late adverse event after intranasal capsaicin treatment (N = 150 approximately till now) nor are there any known to us from the literature, allows us to conclude that intranasal capsaicin is safe to use.

This is supported by data from other medical specialities. Recently intranasal capsaicin cream and civamide solution (the vanilloid receptor agonist cis-capsaicin), in comparable concentrations have been used as a (preventive) treatment during an episodic cluster headache period without local anaesthesia (66, 67). The most common, and of course expected, adverse events included nasal burning and lacrimation. As with our studies, no systemic side-effects were observed. The same accounts for intravesical capsaicin instillations in comparable concentrations for the treatment of interstitial cystitis (68).

Most of our IR patients treated with capsaicin (with or without a trial connection) are still satisfied after a one year follow up and thereby discharged. The few capsaicin treated patients that did come back with a return of their complaints, after having been succesfully discharged one year after the capsaicin treatment, all showed a renewed positive reaction to capsaicin therapy (unpublished data).

In a 1995 review article Y.J Surh and S.S Lee critically examined findings in the literature of studies testing a possible carcinogenic, cocarcinogenic and or anticarcinogenic activity of capsaicin (73). Ingestion of large amounts of capsaicin has been reported to cause histopathological and biochemical changes, including erosion of gastric mucosa and hepatic necrosis (74). Numerous investigations have been conducted, mainly on laboratory animals and ex vivo, to determine the potential mutagenic and carcinogenic activity of capsaicin and chilli pepper, but results are discordant. Whether capsaicin is anticarcinogenic or carcinogenic and what makes this hot substance anti- or carcinogenic still remains unknown (73).

The idea that minute quantities of capsaicin might be carcinogenic seems unlikely to us for it is the natural ingredient of hot-pepper consumed daily by billions of people, especially in Asiatic countries. If there should be even the smallest carcinogenic potency in capsaicin, one might expect a higher incidence of these carcinomas in these countries with a large hot-pepper consumption or in labourers in the 'hot-pepper' industry.

A recent hospital based case-control study conducted in Mexico revealed that high-level consumers of chilli peppers (90-250 mg of capsaicin per day) had an increased risk of gastric cancer compared to low-level chilli pepper consumers (0-29.9 mg of capsaicin per day) (75). Besides the methodologi-

cal considerations one can have with this study, it remains unclear whether capsaicin present in hot chilli-pepper is the major causative factor in the aetiology of gastric cancer in human beings. Other causative factors might be confounders like other hot-chilli ingredients, increased alcohol consumption with the hot meals, etc.
Recapitulating, we don't think that an one-time treatment of five intranasal capsaicin applications at one-hour intervals of less than 1 mg per application, maintains any carcinogenic risk what so ever.

SPECULATIONS ON FUTURE RESEARCH

Capsaicin therapy
In our opinion the one day capsaicin treatment, described in Chapter IV, marks a step forward in the therapy of IR patients not reacting to topical steroids. Due to the relative ease to administer this one day capsaicin treatment at the outpatient clinic we are also hopeful that others, notably the first and second echelon, will introduce this treatment in their practice and will corroborate our findings in the near future.
Another interesting capsaicin treatment regimen is the one suggested by Eberle (69) in which patients apply a low-dose intranasal capsaicin solution 3 times a day during a period of 4 weeks at home without local anaesthesia. He found a marked reduction in symptoms (although not quantified) without significant side effects in 61 IR patients. Unfortunately, in a recent double-blind placebo controlled pilot study, we did not find a positive treatment effect after a 6 week treatment period of 3 times a day intranasal self administration of a capsaicin 10^{-6} M solution (unpublished data). Maybe we did not find any positive treatment effect because of a too low capsaicin concentration (0.0273 microgram per actuation in contrast with Eberle who used a capsaicin spray of 0.125 microgram per actuation) . We chose this low concentration because we wanted to conduct the study in a placebo controlled way without local anaesthesia. Further research has to show whether intranasal self administration of a low dose capsaicin solution is preferable to the present one day capsaicin treatment regimen and, in that case, which capsaicin concentration suites best with regard to therapeutic effect and patient tolerance. Also a combination of previous schemes can be imagined.

One might also consider to use capsaicin as a treatment for IR patients favourably reacting to topical steroids. In view of our practical capsaicin application schedule one might speculate about a double-blind placebo controlled trial comparing this one day capsaicin application regimen with daily local corticosteroids for a period of for instance 6 weeks. It would be very interesting to know whether this group also favourably reacts to capsaicin or not and whether there are histological differences between the group of IR patients favourably and unfavourably reacting to local corticosteroids.

Pathophysiology

In view of our repeatedly negative histological study results regarding the NANC-hypothesis in IR (neurogenic staining) it may be more worthwhile to conduct quantitative studies into neuropeptide content of the nasal mucosa. Like Lacroix (23) one can use radioimmunoassay techniques to measure neuropeptide concentrations (like SP, CGRP and VIP) in weighed homogenized rapidly frozen samples of nasal mucosa. It would be very interesting to perform these measurements on nasal mucosa samples of IR patients before and after capsaicin provocation and before and after treatment. Results may well shed new light on the validity of the NANC-hypothesis in IR and the working mechanism of capsaicin.

Also, as mentioned before, much can be expected from the recent discovery of a range of receptors which respond to capsaicin, menthol, and temperature and their expression in subsets of sensory nerve fibres. More insight into sensory dysfunction at the receptor level provides new prospect towards advancing our understanding and treatment of IR and the more precise modes of action of capsaicin (63-65).

With regards to the possible "hyperesthesia at the CNS level" one might speculate on performing function MRI or PET scan studies before and after provocation in IR patients and controls. Whether these neuro-activity image techniques are already sensitive enough to measure differences at such a specific level may be wondered but it would be an interesting exploration into the role of the CNS in IR, although expensive.

PROSPECTS

Now that one of the problems concerning further research into IR is tackled, namely the developing of a commonly agreed on nomenclature (1), hopefully funding agencies and research groups are more willing to put extra effort into the examination of the pathophysiology (-ies) of IR in the near future. Although fundraising for this topic has shown to be very difficult in recent years. Most charitable institutions and non-profit organisations rather spend their money on 'more important, better measurable' medical problems than on people suffering from some nasal catarrh. Hereby doing injustice to the millions of sufferers who are left without any understanding of the reasons behind their complaints. It also surpasses the fact of the poor quality of life status in IR patients, comparable with those suffering from asthma (70), and the impact of nasal complaints on social life (71S).
The fact that capsaicin is a free obtainable product that cannot be patented, makes it uninteresting for the pharmaceutical industry to sponsor research into this field. The advantage of this is that every local/hospital pharmacist should be able to deliver an intranasal capsaicin solution. This makes the introduction of capsaicin therapy for IR patients in daily practice a realistic goal, independent of this same industry.

CONCLUDING REMARKS:

- According to our histological outcome parameters we were able to select a well defined group of IR patients.
- NARES patients were not included in our IR patient groups, probably because of a good response of these patients to intranasal corticosteroids.
- Local, intranasal capsaicin is a safe and effective therapy in IR.
- Five intranasal capsaicin applications at 1-h intervals on a single day are at least as effective in treating idiopathic rhinitis as five capsaicin applications once every second or third day and with that, more patient and physician friendly.
- Intranasal CDA provocation results in increased mucus production and

nasal blockage in a dose-dependent manner in IR patients and is a suitable tool for monitoring treatment effect in IR.
- Inflammatory cells are not involved in the pathophysiology of IR.
- Inflammatory cell densities and overall neurogenic staining of the nasal mucosa are not affected by intranasal capsaicin provocation after local anaesthesia.
- The etiology of IR is still unclear and will probably be multiple in view of the fact that IR is a diagnosis per exclusionem.

REFERENCES

1 . Bousquet J, van Cauwenberge P, Khaltaev N. Allergic Rhinitis and its impact on asthma (ARIA). J Allergy Clin Immunol 2001;108(5 part 2):S147-334.
2. Blom HM, van Rijswijk JB, Garrelds IM, Mulder PG, Timmermans T, Gerth van Wijk R. Intranasal capsaicin is efficacious in non-allergic, non-infectious perennial rhinitis. A placebo-controlled study. Clin Exp Allergy 1997;27(7):796-801.
3. Sanico A, Togias A. Noninfectious, nonallergic rhinitis (NINAR): considerations on possible mechanisms. Am J Rhinol 1998;12(1):65-72.
4. Wilde AD, Cook JA, Jones AS. The nasal response to isometric exercise in non-eosinophilic intrinsic rhinitis. Clin Otolaryngol 1996;21(1):84-6.
5. Fang SY, Shen CL. Neuropeptide innervation and neuroendocrine cells in allergic rhinitis and chronic hypertrophic rhinitis. Clin Exp Allergy 1998;28(2):228-32.
6. Mikaelian AJ. Vasomotor rhinitis. [Review]. Ear Nose Throat J 1989;68(3):207-10.
7. Blom HM, Godthelp T, Fokkens WF, Kleinjan A, Mulder PGM, Rijntjes E. The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with a nonallergic noninfectious perennial rhinitis. J Allergy Clin Immunol 1997;100(..):739-47.
8. Mygind N, Weeke B. Allergic and vasomotor rhinitis: Clinical aspects. Copenhagen: Munksgaard; 1985.
9. Philip G, Togias AG. Nonallergic rhinitis. Pathophysiology and models for study. Eur Arch Otorhinolaryngol Suppl 1995;1(3):S27-32.
10. Carney AS, Jones NS. Idiopathic rhinitis: idiopathic or not? Clin Otolaryngol 1996;21(3):198-202.

11. Shatkin JS, Delsupehe KG, Thisted RA, Corey JP. Mucosal allergy in the absence of systemic allergy in nasal polyposis and rhinitis: a meta-analysis. Otolaryngol Head Neck Surg 1994;111(5):553-6.
12. Knani J, Campbell A, Enander I, Peterson CG, Michel FB, Bousquet J. Indirect evidence of nasal inflammation assessed by titration of inflammatory mediators and enumeration of cells in nasal secretions of patients with chronic rhinitis. J Allergy Clin Immunol 1992;90(6 Pt 1):880-9.
13. Abe Y, Takeda N, Irifune M, Ogino S, Kalubi B, Imamura I, et al. Effects of capsaicin desensitization on nasal allergy-like symptoms and histamine release in the nose induced by toluene diisocyanate in guinea pigs. Acta Otolaryngol 1992;112(4):703-9.
14. Terrahe K. [Hyperreflectoric rhinopathy]. [German]. Hno 1985;33(2):51-7.
15. Braunstein G, Buvry A, Lacronique J, Desjardins N, Frossard N. Do nasal mast cells release histamine on stimulation with substance P in allergic rhinitis? [see comments]. Clin Exp Allergy 1994;24(10):922-9.
16. Hua XY, Jinno S, Back SM, Tam EK, Yaksh TL. Multiple mechanisms for the effects of capsaicin, bradykinin and nicotine on CGRP release from tracheal afferent nerves: role of prostaglandins, sympathetic nerves and mast cells. Neuropharmacology 1994;33(10):1147-54.
17. Ciprandi G. Treatment of nonallergic perennial rhinitis. Allergy 2004;59 (Suppl. 76):16-23.
18. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. J Allergy Clin Immunol 1992;89(4):877-83.
19. Fokkens WJ, Holm AF, Rijntjes E, Mulder PG, Vroom TM. Characterization and quantification of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. Int Arch Allergy Appl Immunol 1990;93(1):66-72.
20. Braunstahl GJ, Overbeek SE, Fokkens WJ, Kleinjan A, McEuen AR, Walls AF, et al. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. Am J Respir Crit Care Med 2001;164(5):858-65.
21. Wolf G. [New aspects in the pathogenesis and therapy of hyperreflexive rhinopathy]. Laryngol Rhinol Otol (Stuttg) 1988;67(9):438-45.
22. Lundblad L, Saria A, Lundberg JM, Anggard A. Increased vascular permeability in rat nasal mucosa induced by substance P and stimulation of capsaicin-sensitive trigeminal neurons. Acta Otolaryngol 1983;96(5-6):479-84.
23. Lacroix JS, Kurt AM, Pochon N, Bretton C, Lunberg JM, Deshusses J. Neutral

endopeptidase activity and concentration of sensory neuropeptide in the human nasal mucosa. Eur Arch Otorhinolaryngol 1995;252:465-468.
24. Lacroix JS, Buvelot JM, Polla BS, Lundberg JM. Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy 1991;21(5):595-600.
25. Lacroix JS, Pochon N, Lundberg JM. Increased concentration of sensory neuropeptide in the nasal mucosa from patients with non-allergic chronic rhinitis. In: The New frontiers of otorhinolaryngology in europe; 1992; Sorrento, Italy: Monduzzi Editore S.p.A., Italy; 1992. p. 59-63.
26. Lundblad L, Lundberg JM, Brodin E, Anggard A. Origin and distribution of capsaicin-sensitive substance-P-immunoreactive nerves in the nasal mucosa. Acta Otolaryngol 1983;96:485-493.
27. Uddman R, Alumets J, Densert O, Hakansson R, Sundler F. Occurence and distrbution of VIP nerves in the nasal mucosa and the tracheobronchial wall. Acta Otolaryngol 1978;85:443-448.
28. Baraniuk JN. Neuropeptides. Am J Rhinol 1998;12:9-16.
29. Baraniuk JN, Kaliner MA. Neuropeptides and nasal secretion. J Allergy Clin Immunol 1990;86(4 Pt 2):620-7.
30. Svensson C, Andersson M, Greiff L, Persson CG. Nasal mucosal endorgan hyperresponsiveness. Am J Rhinol 1998;12(1):37-43.
31. Anggard A. Basic mechanisms in autonomic nervous responses in specific and nonspecific nasal hyperreactivity. Acta Otolaryngol 1993;113:394-396.
32. Sanico AM, Atsuta S, Proud D, Togias A. Dose-dependent effects of capsaicin nasal challenge: in vivo evidence of human airway neurogenic inflammation. J Allergy Clin Immunol 1997;100(5):632-41.
33. Wolf G, Anderhuber W, Hauser-Kronberger C, Saria A. [Treatment of nonspecific hyper-reflectory rhinopathy (vasomotor rhinitis) with capsaicin]. Laryngorhinootologie 1995;74(5):289-93.
34. Riechelmann H, Davris S, Bader D. [Treatment of perennial non-allergic rhinopathy with capsaicin]. Hno 1993;41(10):475-9.
35. Marabini S, Ciabatti PG, Polli G, Fusco BM, Geppetti P. Beneficial effects of intranasal applications of capsaicin in patients with vasomotor rhinitis. Eur Arch Otorhinolaryngol 1991;248(4):191-4.
36. Sessle BJ. Neural mechanisms and pathways in craniofacial pain. Can J Neurol Sci 1999;26 Suppl 3:S7-11.
37. Wilder-Smith OH, Tassonyi E, Arendt-Nielsen L. Preoperative back pain is associated with diverse manifestations of central neuroplasticity. Pain 2002;97(3):189-194.

38. Jensen TS. Recent advances in pain research: implications for chronic headache. Cephalalgia 2001;21(7):765-769.
39. D'Amato G, Liccardi G, D'Amato M, Cazzola M. The role of outdoor air pollution and climatic changes on the rising trends in respiratory allergy. Respir Med 2001;95(7):606-611.
40. Roemer W, Clench-Aas J, Englert N, al e. Inhomogeneity in response to air pollution in European children (PEACE project). Occup Environ Med 1999;56(2):86-92.
41. Rossi OV, Kinnula VL, Tienari J, Huhti E. Association of severe asthma attacks with weather, pollen, and air pollutants. Thorax 1993;48(3):244-248.
42. Braat JPM, Mulder PG, Duivenvoorden HJ, Gerth van Wijk R, Rijntjes E, Fokkens WJ. Pollutional and meteorological factors are closely related to complaints of non-allergic, non-infectious perennial rhinitis patients: a time series model. Clin Exp Allergy 2002;32(5):690-697.
43. Boezen HM, van der Zee SC, Postma DS, al e. Effects of ambient air pollution on upper and lower respiratory symptoms and peak expiratory flow in children. Lancet 1999;353(9156):874-878.
44. Koltai PJ. Effects of air pollution on the upper respiratory tract of children. Otolaryngol Head Neck Surg 1994;1:9-11.
45. Mygind N. Allergic and non-allergic rhinitis. Clinical aspects. 1 ed. Copenhagen: Munksgaard; 1993.
46. Folinsbee LJ. Does nitrogen dioxide exposure increase airways responsiveness? Toxicol Ind Health 1992;8(5):273-283.
47. Graham D, Henderson F, House D. Neutrophil influx measured in nasal lavages of humans exposed to ozone. Arch Environ Health 1988;43(228-233).
48. Coggins CR, Fouillet XL, Lam R, Morgan KT. Cigarette smoke induced pathology of the rat respiratory tract: a comparison of the effects of the particulate and vapour phases. Toxicology 1980;16:83-101.
49. Gerth van Wijk R. Perennial allergic rhinitis and nasal hyperreactivity. Am J Rhinol 1998;12:33-35.
50. Gerth van Wijk R, van Toorenenbergen AW, Zijlstra FJ, al e. Nasal hyperreactivity and its effect on early and late sequelae of nasal challenge with house-dust mite extract. Allergy Proc 1993;14:273-281.
51. de Graaf-in 't Veld C, Garrelds IM, Koenders S, Gerth van Wijk R. Relationships between nasal hyperreactivity, mediators, and eosinophils in patients with perennial allergic rhinitis and controls. Clin Exp Allergy 1996;26:903-909.
52. de Graaf-in 't Veld C, Garrelds IM, van Toorenenbergen AW, Gerth van Wijk R. Nasal responsiveness to allergen and histamine in patients with perennial allergic rhinitis with and without a late phase reaction. Thorax 1997;52:143-148.

53. Wang DY, Clement P. Pathogenic mechanisms underlying the clinical symptoms of allergic rhinitis. Am J Rhinol 2000;14(5):325-333.
54. Braunstahl GJ, Overbeek SE, Kleinjan A, Prins JB, Hoogsteden HC, Fokkens WJ. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. J Allergy Clin Immunol 2001;107:469-476.
55. Braat JP, Mulder PG, Fokkens WJ, Gerth van Wijk R, Rijntjes E. Intranasal cold dry air is superior to histamine challenge in determining the presence and degree of nasal hyperreactivity in nonallergic noninfectious perennial rhinitis. Am J Respir Crit Care Med 1998;157(6 Pt 1):1748-55.
56. Braat JPM, Fokkens WJ, Mulder PG, Kianmaneshrad N, Rijntjes E, Gerth van Wijk R. Forced expiration through the nose is a stimulus for NANIPER but not for controls. Rhinology 2000;38(4):172-176.
57. Kubo N, Kumazawa T. Functional disturbances of the autonomic nerve in nasal hyperreactivity: an up-date review. Acta Otolaryngol. 1993;113:394-396.
58. Jancso N, Jancso-Gabor A, Szolcsanyi J. Direct evidence of neurogenic inflammation and its prevention by denervation and pretreatment with capsaicin. Br J Pharmacol Chemotherapy 1967(31):138-51.
59. Holzer P. Local effector functions of capsaicin sensitive nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 1988;24:739-768.
60. Baraniuk JN. Sensory, parasympathetic, and sympathetic neural influences in the nasal mucosa. J Allergy Clin Immunol 1992;90(6 Pt 2):1045-50.
61. Holzer P. Capsaicin;cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 1991;43(2):143-201.
62. Kitajiri M, Kubo N, Ikeda H, Sato K, Kumazawa T. Effects of topical capsaicin on autonomic nerves in experimentally-induced nasal hypersensitivity. An immunocytochemical study. Acta Otolaryngol Suppl 1993;500(2):88-91.
63. Anand P. Capsaicin and menthol in the treatment of itch and pain: recently cloned receptors provide the key. Gut 2003;52(9):1233-1235.
64. Rawlingson A, Gerard NP, Brain SD. Interactive contribution of NK(1) and kinin receptors to the acute inflammatory oedema observed in response to noxious heat stimulation: studies in NK(1) receptor knockout mice. Br J Pharmacol 2001;134(8):1805-1813.
65. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway [see comments]. Nature 1997;389(6653):816-24.
66. Marks DR, Rapoport A, Padla D, Weeks R, Rosum R, Sheftell F, et al. A

double-blind placebo-controlled trial of intranasal capsaicin for cluster headache. Cephalalgia 1993;13(2):114-116.
67. Saper JR, Klapper J, Mathew NT, Rapoport A, Phillips SB, Bernstein JE. Intranasal civamide for the treatment of episodic cluster headaches. Arch Neurol 2002;59(6):990-994.
68. Fagerli J, Fraser MO, de Groat WC, Chancellor MB, Flood HD, Smit Jordan ML. Intravesical capsaicin for the treatment of interstitial cystitis: pilot study. Can J Urol 1999;6(2):737-744.
69. Eberle L, Gluck U. [Clinical experiences with local capsaicin treatment of chronic rhinopathy]. Hno 1994;42(11):665-9.
70. Bousquet J, Knani J, Dhivert H, et al. Quality of life in asthma. I. Internal consistency and validity of the SF-36 questionnaire. Am J Respir Crit Med 1994;149(2 part 1):371-375.
71. Juniper EF. Impact of upper respiratory allergic diseases on quality of life. J Allergy Clin Immunol 1998;101(2 Pt 2):S386-91.
72. Gerth van Wijk R, Terreehorst IT, Mulder PG, Garrelds IM, Blom HM, Popering S. Intranasal capsaicin is lacking therapeutic effect in perennial allergic rhinitis to house dust mite. A placebo-controlled study. Clin Exp Allergy 2000; 30 (12), 1792-1798.
73. Surh Y-J, Lee SS. Capsaicin in Hot Chili Pepper: Carcinogen, Co-carcinogen or Anticarcinogen? Fd Chem Toxic 1996; 34(3), 313-316.
74. Monsereenusorn Y, Kongsamut S, Pezella PD. Capsaicin - a literature survey. CRC Critical Reviews in Toxicology 1982; 10, 321-339.
75. Lopez-Carillo L, Lopez-Cervantes M, Robles-Diaz G, Ramirez-Espitia A, Mohar-Betancourt A, Meneses-Garcia A, Lopez-Vidal Y, Blair A. Capsaicin consumption, Helicobacter pylori positivity and gastric cancer in Mexico. Int J Cancer 2003; 106(2), 277-282.

Summary
Samenvatting

Summary

In chapter I the currently known causes for nonallergic noninfectious rhinitis and possible treatments are summarised. Also possible pathophysiological mechanisms underlying idiopathic rhinitis (IR) are discussed.
In chapter II the aims of the studies are presented. This thesis comprises studies aimed at the therapeutic potential and safety of intranasal capsaicin in IR patients, developing a patient and physician friendly, effective intranasal capsaicin treatment regimen for IR and the histological analysis of the nasal mucosa of IR patients in order to find an explanation for the therapeutic effect of capsaicin and the underlying pathophysiology of IR.
In chapter III the efficacy of intranasal capsaicin spray in the treatment of IR patients was studied. Several authors described capsaicin, the pungent substance in red-pepper, as an efficacious therapy for IR. Repeated intranasal capsaicin application induces peptide depletion and specific degeneration of the unmyelinated sensory C-fibers in the nasal mucosa. We performed a double-blind placebo (NaCl 0.9%) controlled study with 25 IR patients. Daily record charts (DRC) and visual analogue scales (VAS) were used for clinical evaluation. Nasal lavages were obtained before, during and after treatment. There was a significant and long-term reduction in the VAS scores in the capsaicin group. No significant difference was found between the placebo and capsaicin treated groups for the mean group concentrations of leukotriene (LT) C_4 / D_4 / E_4, prostaglandin D_2 (PGD_2), and tryptase. The levels of mast cell mediators, tryptase and PGD_2, and leukotrienes, mediators derived from a variety of inflammatory cells, were low at baseline and comparable with levels observed in nasal lavages obtained from normals. As involvement of inflammation could not be demonstrated, it is not surprising that capsaicin has no effect on inflammatory mediators. This suggests that inflammatory cells do not play a major part in the pathogenesis of IR.
In chapter IV we conducted a double-blind double-dummy parallel group trial to determine whether a more practical capsaicin application schedule is equally effective as the one described in chapter III. In daily practice, this application regimen of intranasal capsaicin application once every second or third day for a total of 7 days proved to be impractical because of the large number of visits required in a short period of time. Thirty patients were

randomized into two different treatment regimens: one group received capsaicin five times the first day at one-hour intervals. This was followed by a placebo dummy once every second or third day for a total of five treatments 2 weeks after the capsaicin application (group A). The other group (B) received the placebo dummy five times on the first day followed by capsaicin once every second or third day for a total of five treatments 2 weeks after the placebo application. The VAS scores for overall nasal symptoms, rhinorrhea and nasal blockage showed significant decrease after the start of treatment in both groups, with a significantly steeper decrease in group A. A significant reduction in cold dry air dose responsiveness was also found up to 9 months after therapy in both groups, reflecting a decrease in nasal hyperreactivity. No significant changes in safety data (smell, blood pressure, heart rate) were found. We conclude that local capsaicin nasal spray significantly reduces nasal complaints in IR patients and that five treatments of capsaicin on a single day is at least as effective as five treatments of capsaicin in 2 weeks, and even more effective in the reduction of nasal complaints measured with VAS. We also conclude that intranasal capsaicin seems safe to use.

In chapter V nasal mucosa inflammatory cell densities of IR patients are studied and compared with normals. Mucosal inflammatory cellular infiltrates are correlated with nasal complaints in symptomatic allergic rhinitis. Some authors suggest inflammation of neurogenic or immunogenic nature as the underlying pathophysiology for IR. We examined whether inflammatory cells are involved in the pathogenesis of IR. Nasal biopsies were taken of 65 patients with significant nasal complaints and 20 controls without nasal complaints. Inflammatory cells were quantified, using monoclonal antibodies directed against lymphocytes, antigen presenting cells, eosinophils, macrophages, monocytes, mast cells and other IgE-positive cells. No significant differences were found, for any cell, between IR patients and controls. We conclude that inflammatory cells do not seem to play an important role in this meticulously characterised group of IR patients.

In chapter VI the long term effects of capsaicin spray on the nasal mucosa are studied. Capsaicin has been shown previously to reduce nasal complaints in patients with IR. Proposed pathophysiologic mechanisms for IR include a chronic inflammatory disorder of antigenic or neurogenic nature as well as the possibility of a functional neuronal disorder. We hypothesized that the

beneficial effect of capsaicin might be the result of a down regulation of inflammation (by a reduction of inflammatory cells) or through a modulation of neural tissue density. Patients were treated with either a placebo or capsaicin spray solution once every second or third day for a total of seven treatments. Both sides were treated each visit. Biopsies were taken before, 2 weeks after, 3 months after and 9 months after the treatment period. Immunohistochemical staining of the biopsy specimen was performed to ascertain the effect of treatment on immunocompetent cell densities (quantitative) and neural tissue densities (semiquantitative) in the nasal mucosa. Nasal complaints were significantly reduced in the capsaicin treated group (Chapter III). The number of lymphocytes, antigen presenting cells, eosinophils, macrophages, monocytes, mast cells and other IgE-positive cells did not significantly differ between the capsaicin and the placebo group. No significant differences between both groups were found in pan-neurogenic staining of nasal mucosa using neurofilament and synaptophysine. To conclude capsaicin intranasal spray does significantly improve nasal symptomatology in IR patients (shown previously), without affecting cellular homeostasis or overall neurogenic staining upto 9 months after treatment. Immunocompetent cells are not involved in IR.

In chapter VII we tried to get more insight into the pathophysiologic mechanism of IR and the direct mode of action of capsaicin on nasal mucosa cell counts and neurogenic staining. To our knowledge data concerning the provocation effect of capsaicin on nasal cellular homeostasis in humans is limited to lavage studies following a single capsaicin application in allergic patients and healthy controls. We performed a double-blind placebo controlled nasal biopsy study in 30 strictly selected and well-defined IR patients challenged with either capsaicin or placebo. Biopsies were taken at baseline 2 weeks before provocation and 15 minutes and 1 hour after a single provocation with capsaicin or placebo. The cell densities of CD3, CD8, CD25, C-Kit, chymase, tryptase, BB1, IgE and BMK 13 and the neuronal staining with synaptophysine, neurofilament and VRL-1 were studied in both layers of the nasal mucosa. No significant difference in nasal mucosa cell counts and neurogenic staining were found 15 minutes and 1 hour after provocation. Only for CD3 in the epithelium 1 hour after provocation a significant higher cell count was found in the capsaicin group using the Mann-Whitney U test.

Due to multiple testing this p-value of 0.011 for CD3 could have been easily caused by chance. We conclude that capsaicin, after local anaesthesia of the nasal mucosa, does not affect cellular homeostasis or neurogenic staining 15 minutes and 1 hour after nasal provocation in this double-blind placebo controlled biopsy study. This strengthens us in our idea that inflammatory cells do not play a role in the mode of action of capsaicin and the aetiology of IR.

Samenvatting

In Hoofdstuk I worden de momenteel bekende oorzaken en behandelingen van niet allergische, niet infectieuze perenniale rhinitis beschreven. Ook worden mogelijk aan idiopathische rhinitis (IR) ten grondslag liggende oorzaken besproken en bediscussieerd.
In Hoofdstuk II worden de doelstellingen van dit proefschrift beschreven. Dit proefschrift omvat studies naar het therapeutisch effect en veiligheid van intranasale capsaicine spray bij IR patiënten, het ontwikkelen van een patiënt en behandelaar vriendelijk, effectief capsaicine toedieningsschema voor de behandeling van IR en de histologie van neusslijmvlies biopsieën van IR patiënten ter verklaring van het therapeutisch effect van capsaicine bij IR en de aan IR ten grondslag liggende pathofysiologie.
In Hoofdstuk III wordt het therapeutisch effect van capsaicine spray bij IR patiënten bestudeerd. Capsaicine, het hete bestanddeel van rode peper, wordt door verschillende auteurs beschreven als een effectief middel bij IR. Herhaalde intranasale capsaicine applicaties induceren neuropeptide depletie en specifieke degeneratie van de niet gemyeliniseerde sensibele C-afferente vezels in het neusslijmvlies. Bij 25 patiënten werd een dubbelblinde placebo (NaCl 0.9%) gecontroleerde studie verricht. Dagkaarten en visual anlogue scales (VAS) werden gebruikt voor klachtenregistratie en klinische evaluatie. Voor, en na gedurende behandeling werden neuslavages verricht. Er werd een lang aanhoudende, significante afname van neusklachten gevonden, gemeten met VAS, in de met capsaicine behandelde groep. Er werd geen significant verschil gevonden tussen de met placebo en capsaicine behandelde groep voor de gemiddelde groep concentraties van leukotrienen C4/D4/E4, prostaglandine D2 en tryptase. De uitgangsconcentraties van mestcel mediatoren, tryptase en prostaglandine D2, en leukotrienen, mediatoren afkomstig van een verscheidenheid aan inflammatoire cellen, waren laag en vergelijkbaar met concentraties gevonden in neusspoelingen bij normale controles. Daar betrokkenheid van inflammatie niet kon worden aangetoond, is het niet verwonderlijk dat capsaicine geen effect heeft op ontstekingsmediatoren. Dit suggereert dat inflammatoire cellen geen grote rol spelen in de pathogenese van IR.
In Hoofdstuk IV werd een dubbelblinde dubbeldummie parallelle groepen

studie verricht, om uit te zoeken of een praktischer capsaicine toedieningsschema even effectief zou zijn als die beschreven in Hoofdstuk III. In de dagelijkse praktijk blijkt dit toedieningsschema van intranasale capsaicine spray toediening iedere tweede of derde dag voor een totaal van 7 behandelingen onpraktisch door de vele bezoeken in een korte periode. Dertig patiënten werden gerandomiseerd over 2 behandelgroepen: de ene groep werd de eerste dag vijf keer, om het uur, behandeld met capsaicine. Twee weken hierna volgde behandeling met een placebo dummie een keer per dag iedere tweede of derde dag voor een totaal van 5 behandelingen (groep A). De andere groep (B) werd eerst behandeld met de placebo dummie vijf keer op de eerste dag, 2 weken later gevolgd door capsaicine behandeling een keer per dag iedere tweede of derde dag voor een totaal van 5 behandelingen. De VAS scoren voor algemene neusklachten, loopneus en neusverstopping lieten een significante verbetering zien na aanvang van de therapie in beide groepen, met een significant sterkere verbetering in groep A. Ook werd er een significante afname van de reactie op koude droge lucht provocatie gevonden tot en met 9 maanden na behandeling in beide groepen, wijzend op een afname in nasale hyperreactiviteit. Er werden geen significante veranderingen gevonden in de onderzochte veiligheidsparameters (reuk, bloeddruk, hartslag). Wij concluderen dat capsaicine neusspray de neusklachten van IR patiënten significant vermindert en dat vijf capsaicine behandelingen op een dag minstens zo effectief zijn als vijf capsaicine behandelingen in 2 weken, en zelfs effectiever in de vermindering van neusklachten gemeten met VAS. We concluderen ook dat de behandeling met intranasale capsaicine spray veilig lijkt.

In Hoofdstuk V worden de dichtheden van ontstekingscellen in neusslijmvlies van IR patiënten bestudeerd en vergeleken met die in normale controles. Infiltratie van ontstekingscellen in neusslijmvlies correleert met neusklachten in symptomatische allergische rhinitis patiënten. Inflammatie van neurogene dan wel immunogene aard wordt door sommige auteurs als het onderliggend lijden in IR gezien. Wij onderzochten of ontstekingscellen betrokken zijn bij de pathogenese van IR. Van 65 patiënten met significante neusklachten en 20 normale controles zonder neusklachten werden neusslijmvlies biopsieën genomen. Met behulp van monoklonale antistoffen gericht tegen lymfocyten, antigeen presenterende cellen, eosinofielen, macrofagen, monocyten,

mestcellen en andere IgE-positieve cellen werden de ontstekingscellen gekwantificeerd. Voor geen enkele cel werden er significante verschillen gevonden tussen de IR patiënten en de normale controles. Wij concluderen dat ontstekingscellen geen belangrijke rol lijken te spelen in deze streng geselecteerde groep van IR patiënten.
In Hoofdstuk VI worden de lange termijn effecten van capsaicine spray op het neusslijmvlies van IR patiënten bestudeerd. Van capsaicine is aangetoond dat het afname van neusklachten geeft bij patiënten met IR (zie ook Hoofdstuk III en IV). Veronderstelde pathogenetische mechanismen omvatten een chronisch inflammatoire aandoening van antigene dan wel neurogene aard of wel een functioneel neurologisch lijden. Wij onderzochten of het therapeutische effect van capsaicine mogelijk veroorzaakt wordt door een afname van inflammatie (vermindering van ontstekingscellen) of door een verandering van de dichtheid in zenuwweefsel in het neusslijmvlies. Patiënten werden behandeld met placebo of capsaicine spray (0.15 mg capsaicine per neusgat) iedere tweede of derde dag voor een totaal van 7 behandelingen. Elk bezoek werden beide zijden van de neus behandeld. Voorafgaand aan en 2 weken, 3 maanden en 9 maanden na de behandelingsperiode werd er een neusslijmvlies biopsie genomen. De coupes van deze biopsieën werden immunohistochemisch gekleurd om het effect van de behandeling op immunocompetente cel dichtheden (kwantitatief) en zenuwweefsel dichtheid (semi-kwantitatief) te bepalen. De neusklachten waren significant verbeterd in de met capsaicine behandelde patiënten groep zoals beschreven in Hoofdstuk III. Het aantal lymfocyten, antigeen presenterende cellen, eosinofielen, macrofagen, monocyten, mestcellen en andere IgE-positieve cellen lieten geen significant verschil zien tussen de met capsaicine en placebo behandelde groep. Ook werden er geen significante verschillen gevonden tussen beide groepen na pan-neurogene kleuringen van de neusslijmvlies biopten met behulp van de neuromarkers neurofilament en synaptofysine. Concluderend geeft capsaicine een significante verbetering van neusklachten in IR patiënten (eerder aangetoond), zonder effect op inflammatoire cellen in, of pan-neurogene kleuringen van neusslijmvlies biopten tot en met 9 maanden na behandeling. Immunocompetente cellen zijn niet betrokken bij IR.
In Hoofdstuk VII hebben we getracht meer inzicht te krijgen in de pathofysiologische mechanismen van IR en de directe werking van capsaicine

op neusslijmvlies cel tellingen en neurogene kleuring. Voor zover wij na kunnen gaan zijn studies naar het provocatie effect van capsaicine op cel homeostase in het neusslijmvlies van mensen beperkt gebleven tot neuslavage studies na een enkele capsaicine spray applicatie bij allergische patiënten en gezonde controles. Wij verrichtten een dubbelblinde placebo gecontroleerde studie naar neusslijmvlies biopsieën van 30 streng geselecteerde en goed gedefinieerde IR patiënten die werden geprovoceerd met capsaicine of placebo. Twee weken voor en 15 minuten en 1 uur na een enkele capsaicine of placebo provocatie werden de neusslijmvlies biopsieën afgenomen. De cel dichtheden van cellen aangekleurd op CD3, CD8, CD25, C-Kit, chymase, tryptase, BB1, IgE en BMK 13 en zenuwweefsel dichtheid na neurogene kleuring met synaptofysine, neurofilament en VRL-1 werden bestudeerd in beide lagen van het neusslijmvlies. Er werden geen significante verschillen gevonden in cel aantallen of neurogene kleuring in de biopsieën afgenomen 15 minuten en 1 uur na provocatie. Alleen voor CD3 in het epitheel 1 uur na provocatie werd een significant hoger cel aantal gevonden in de met capsaicine geprovoceerde groep met behulp van de Mann-Whitney U statistische test. Gezien het multipele testen in deze studie zou deze p-waarde van 0.011 heel wel door toeval veroorzaakt kunnen zijn. Wij concluderen dat capsaicine, na lokale verdoving van het neusslijmvlies, geen effect heeft op cel homeostase of neurogene kleuring 15 minuten en 1 uur na neusprovocatie in deze dubbelblinde placebo gecontroleerde biopsie studie. Dit versterkt ons idee dat ontstekingscellen geen rol spelen in het werkingsmechanisme van capsaicine en de etiologie van IR.

List of abbreviations

NANIPER	nonallergic noninfectious perennial rhinitis
IR	idiopathic rhinitis
NARES	nonallergic rhinitis with eosinophilia syndrome
CGRP	calcitonin-gene-related-peptide
SP	substance-P
DRC	daily record chart
VAS	visual analogue scale
UPSIT	University of Pennsylvania smell identification test
PNIF	peak nasal inspiratory flow
MCA	minimal cross-sectional area
MMCA	mean of the minimal cross-sectional area
TMMCA	sum of right and left MMCA
CI	confidence interval
SD	standard deviation
BP	blood pressure
HR	heart rate
VRL-1	vaniloid receptor ligand -1
CD	cluster of differentiation

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Curriculum Vitae

Levensloop

Jeroen Bernard van Rijswijk werd op 14 april 1971 te Berekum, Ghana geboren. In 1989 haalde hij het V.W.O. diploma aan de Rijks Scholengemeenschap in Middelharnis. Hetzelfde jaar begon hij aan de studie Chemische Technologie te Delft. Van 1990 tot en met 1997 studeerde hij geneeskunde aan de Erasmus Universiteit Rotterdam. In augustus 1997 ving hij aan als arts-assistent KNO aan het Dijkzigt Ziekenhuis Rotterdam onder leiding van professor Verwoerd, eerst als AGNIO en later in opleiding, die in oktober 2003 werd afgerond. De basis voor het onderzoek dat resulteerde in dit proefschrift werd gelegd tijdens het geneeskunde afstudeerproject onder leiding van H.M. Blom en verder uitgebouwd tijdens de KNO-assistenten periode.
Sinds januari 2004 is hij naar volle tevredenheid werkzaam als vrij gevestigd KNO-arts, met als aandachtsgebied de esthetische en reconstructieve aangezichtschirurgie, in het van Weel-Bethesda ziekenhuis te Dirksland in associatie met D. van Hasselt.
Hij is gehuwd met Nienske Sophie van Rijswijk-Peters.