ORIGINAL ARTICLE

Effect of Fluticasone on Neuropeptides in Nasal Lavage in Persistent Allergic Rhinitis

C Schäper,[1] B Gustavus,[2] B Koch,[1] R Ewert,[1] SB Felix,[1] G Kunkel,[2] O Noga,[2] S Gläser[1]

[1]Department of Internal Medicine B – Cardiology, Intensive Care, Pulmonary Medicine and Infectious Diseases, Universität Greifswald, Greifswald, Germany
[2]Department of Pneumology and Infectious Diseases, Charité-Universitätsmedizin, Berlin, Germany

■ **Abstract**

Objective: Recent guidelines reveal that allergic rhinitis impairs quality of life. Neuropeptides play a central role in allergy-related nasal inflammation. The objective of this study was to analyze the release of neuropeptides (substance P, neurokinin A, and vasoactive intestinal peptide) in nasal lavage and their modification by intranasal fluticasone propionate as an established therapy in patients with allergic rhinitis.

Methods: Eleven patients with proven allergic rhinitis induced by house dust mite were challenged before and after administration of fluticasone propionate nasal spray. Nasal lavage samples were collected after allergen challenge, and neuropeptides were measured using enzyme-linked immunosorbent assay. Values for histamine, protein, and human serum albumin were also recorded. Eight healthy individuals were included as nonatopic controls.

Results: The neuropeptides investigated were detectable in nasal lavage fluid in both patients and controls. Treatment with fluticasone propionate significantly decreased clinical response to allergen challenge ($P<.01$) compared with the controls and led to a decrease in values for substance P, neurokinin A, vasoactive intestinal peptide, histamine release, human serum albumin, and total protein after allergen challenge ($P<.01$).

Conclusions: The demonstration of proinflammatory neuropeptides in NAL and suppression of their release after allergen challenge caused by a topical corticosteroid suggest a role for neuropeptides in allergic inflammation. Diminished release of neuropeptides induced by fluticasone propionate was accompanied by an improvement in the clinical symptoms of patients with persistent allergic rhinitis.

Key words: Substance P. Neurokinin A. Vasoactive intestinal peptide. Persistent allergic rhinitis. Fluticasone propionate. Allergen challenge. Nasal lavage. Neurogenic inflammation.

■ **Resumen**

Objetivo: Las últimas guías de práctica clínica revelan que la rinitis alérgica deteriora la calidad de vida. Los neuropéptidos desempeñan un papel central en la inflamación nasal relacionada con la alergia. El objetivo de este estudio fue analizar la liberación de neuropéptidos (sustancia P, neurocinina A y péptido intestinal vasoactivo) en el lavado nasal y su modificación mediante propionato de fluticasona intranasal como tratamiento establecido en pacientes con rinitis alérgica.

Métodos: Once pacientes con rinitis alérgica demostrada inducida por ácaros del polvo doméstico se sometieron a pruebas de provocación antes y después de administrarles propionato de fluticasona en spray nasal. Se obtuvieron muestras de lavado nasal tras la provocación con alérgenos y se determinaron los neuropéptidos mediante enzimoinmunoanálisis de adsorción. También se registraron los valores de histamina, proteína y seroalbúmina humana. Se incluyeron ocho individuos sanos como controles no atópicos.

Resultados: Los neuropéptidos analizados se detectaron en el líquido de lavado nasal tanto en los pacientes como en los controles. El tratamiento con propionato de fluticasona produjo una disminución significativa de la respuesta clínica a la provocación con alérgenos ($P<0,01$) comparado con los controles, y causó una disminución de los valores de sustancia P, neurocinina A, péptido intestinal vasoactivo, liberación de histamina, seroalbúmina humana y proteína total tras la provocación con alérgenos. ($P<0,01$).

Conclusiones: La presencia demostrada de neuropéptidos proinflamatorios en el líquido de lavado nasal y la supresión de su liberación tras la provocación con alérgenos causada por un corticoesteroide tópico parecen indicar que los neuropéptidos participan en la inflamación alérgica. La disminución de la liberación de neuropéptidos producida por el propionato de fluticasona trajo aparejada una mejora de los síntomas clínicos en los pacientes con rinitis alérgica persistente.

Palabras clave: Sustancia P. Neurocinina A. Péptido intestinal vasoactivo. Rinitis alérgica persistente. Propionato de fluticasona. Provocación con alérgenos. Lavado nasal. Inflamación neurogénica.

Introduction

As recently outlined by the World Health Organization in the Allergic Rhinitis and its Impact on Asthma workshop, allergic rhinitis has a major impact on quality of life [1]. The allergic response to allergen exposure is understood as an inflammatory process. Neurokinins play a role as proinflammatory mediators in airway diseases such as bronchial asthma and allergic rhinitis [2-4]. Airway nerves are involved in the regulation of airway function under physiological conditions and in the pathophysiological events of allergic airway inflammation. Upper airway innervation is anatomically and functionally separated into an autonomic efferent component (including sympathetic and parasympathetic pathways) and a sensory afferent component. Sensory nerve fibers, which are found in high density in the nasal mucosa, are extensively branched, unmyelinated C fibers [5]. These C fibers contain various sensory neuropeptides, including the tachykinin substance P (SP) and neurokinin A (NKA) [6], whereas parasympathetic nerve endings contain vasoactive intestinal peptide (VIP) [7].

Mediators released due to an allergic reaction can modulate the activity of sympathetic, parasympathetic, and sensory neurons, leading in turn to enhanced neurotransmitter release through interaction with sensory nerve receptors, followed by sensory nerve depolarization and nerve impulse generation [8]. Different studies have demonstrated that immune cells are a source of tachykinins such as SP [9].

SP acts on vascular permeability via the NK1 receptor, thus leading to nasal obstruction and mucus secretion from the submucosal glands. NKA is located in the walls of arterioles in human nasal mucosa and acts mainly via NK2 receptors [6]. Forsythe et al [10] demonstrated that SP and NKA induce histamine release from bronchoalveolar mast cells in both nonasthmatic coughers and cough variant asthmatics, thus indicating a neuroimmune interaction within the human lung. In particular, the mast cell degranulation effect generated through the axon reflex is considered to contribute to the development of neurogenic inflammation [11]. Tachykinins are inactivated by enzymatic degradation, mainly by neutral endopeptidase-24.11, which has also been found in several types of nasal tissue cells [12].

VIP coexists with acetylcholine in the parasympathetic neurons in postganglionic parasympathetic nerves surrounding the submucosal glands and vessels [7]. VIP receptors are located on arterial vessels, submucosal glands, epithelial cells, and eosinophils, and may regulate mucociliary clearance [13]. Anti-inflammatory action in asthma has also been attributed to VIP [14], and nasal challenge of allergic humans with antigen and with bradykinin or capsaicin has been shown to induce release of SP and enhance antigen-induced mediator release [15]. However, only a few reports examine the effects of antiallergic agents such as oral antihistamines, which decrease SP and VIP concentrations in nasal lavage fluid in allergic patients with seasonal rhinitis [16]. Few data are available on the effect of NKA on rhinitis, although levels of NKA, which is a more potent bronchoconstrictor than SP, are elevated in the bronchoalveolar lavage fluids of asthmatic patients [17].

Topical corticosteroids containing fluticasone are widely used to relieve nasal symptoms in patients with allergic rhinitis [18].

The aim of this study was to investigate the role of the neuropeptides SP, NKA, and VIP in nasal secretion before and after allergen challenge in allergic patients and healthy volunteers. We also examined the effect of topical fluticasone propionate on symptoms and on the mechanisms of neuropeptide release during the early phase reaction in patients with allergic rhinitis. As house dust mite (HDM) allergen is a relevant perennial allergen, we chose a population of patients with allergic rhinitis due to HDM.

Materials and Methods

Patients and Study Design

Eleven adult patients (6 women; mean [SD] age, 32.2 [12] years [range, 19-65]) with allergic rhinitis due to HDM were compared with 8 healthy controls (4 women; mean age 30 [8] years [range, 19-58]). Antiallergic medication was stopped 6 weeks prior to the study. Patients with inflammatory nasal conditions other than allergic rhinitis were excluded, and no patient was taking immunotherapy or had systemic disease. Allergic rhinitis due to HDM was defined clinically by typical symptoms (obstruction, sneezing, itching, and rhinorrhea) for more than 2 years. Sensitization was confirmed by a positive skin prick test result and serum specific immunoglobulin (Ig) E determination (mean CAP class, 2.6 [0.6]; Pharmacia CAP System, Uppsala, Sweden), a mean total IgE level of 577 (196) kU_A/L, and specific allergen challenge. Five patients had mild bronchial asthma and were using only bronchodilators as needed.

The study design is illustrated in Figure 1.

After the first allergen challenge, patients were treated once daily for 2 weeks with fluticasone propionate nasal spray (100 µg per nostril). All participants gave their informed consent. The study was approved by the local ethics committee of Charité-Humboldt University, Berlin, Germany.
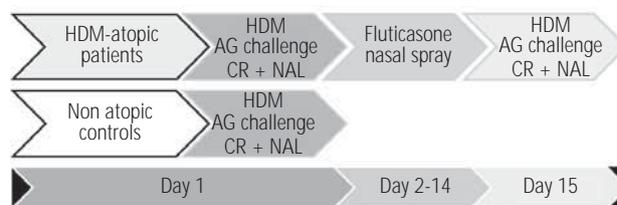

Figure 1. Nasal challenge with house dust mite and antigen and clinical rhinomanometry were performed before (baseline) challenge, 10 minutes after diluent challenge, and 10 minutes after antigen challenge. Nasal lavage for neuropeptide and protein detection was performed at baseline, after diluent challenge and 5, 10, 20, and 30 minutes after antigen challenge. Fluticasone propionate nasal spray was administered at 100 µg/nostril once daily for 14 days. HDM indicates house dust mite; AG, antigen; CR, clinical rhinomanometry; NAL, nasal lavage.

Nasal Allergen Challenge

Nasal symptom score and nasal airway function were assessed before the diluent was administered (baseline), 10 minutes after

(the placebo was albumin-buffered saline), and 10 minutes after the nasal allergen challenge. The authors have previously shown this time point to be suitable for evaluating the changes in score and nasal flow [19]. To analyze the degree of nasal airway obstruction, nasal peak inspiratory flow (cm³/s) and nasal airway resistance (NAR) expressed in Pa (cm³/s) were measured using active anterior rhinomanometry (Rhinotest MP 500, Allergopharma Joachim Ganzer KG, Reinbek, Germany) in each nostril, calculated at a pressure at 150 Pa. The threshold allergen dose for nasal allergen exposure was determined for each allergic individual by dose titration. The mean provocation dose was 500 SBU/nostril in all participants.

The clinical scores for the 4 major symptoms (nasal obstruction, sneezing, itching, and rhinorrhea) were recorded on a 4-point scale: 0, no symptoms; 1, mild symptoms with minimal awareness; 2, moderate/tolerable symptoms; and 3, severe symptoms that were difficult to tolerate and interfered with daily activities and sleeping. The number of sneezes was counted and converted to a score: 0, 0 sneezes; 1, 1-4 sneezes; 2, 5-9 sneezes; and 3, ≥10 sneezes. The total nasal symptom score (0-12) was calculated by adding the points for each patient before and after therapy with fluticasone propionate. The scores before and after the nasal allergen challenge were compared to verify the allergic response.

Nasal Lavage Procedure

Nasal lavage was performed with consecutive saline lavages (4 prewashes), after the diluent was applied (0.1 mL of diluent for the allergen extract), and 5, 10, 20, and 30 minutes after allergen challenge by instillation of 5 mL of normal saline solution (preheated to 37°C) into each nasal cavity through a tube connected to a syringe. The nostrils were sealed with foam rubber. After 10 seconds each fluid sample was collected by repeated aspiration into a polypropylene tube, which was placed on ice immediately. After centrifugation at 1000g for 10 minutes at 4°C and removal of cellular elements, the supernatants were aliquoted for the different assays and stored at –80°C until analysis. All participants underwent challenge with the allergen extract, *Dermatophagoides pteronyssinus* (Allergopharma, Hamburg, Germany; concentration, 5000 SBU/mL). A volume of 0.1 mL of allergen solution (500 SBU/nostril) was sprayed onto the inferior turbinate area of each nostril with a plastic hand-activated nebulizer (De Vilbiss 40; De Vilbiss Co., Somerset, Pennsylvania, USA). The allergen solution was routinely stored in a refrigerator and heated to room temperature before use [19].

The influence of the allergen and of fluticasone propionate was evaluated by comparing the difference between the measurement of mediator concentrations, the intensity of nasal symptoms, nasal airway resistance, and inspiratory peak flow before and after nasal allergen challenge.

Assessment of Mediators

Neuropeptides: Prior to the final challenge, the results of an enzyme-linked immunosorbent assay (ELISA) were evaluated to determine the reliability of the immunoreactivity measurements. Since peptides are easily degraded, a protease inhibitor mix was prepared as follows: phosphoramidon (Sigma R7385, Deisenhofen, Germany) 10 µmol/L, captopril (Sigma C4042) 10 µmol/L, leupeptin (Sigma L2884) 10 µmol/L, puromycin (Sigma P7255) 100 µmol/L, and aprotinin (Sigma A1153) 400 kU/mL. All 3 peptides were measured in duplicate using highly specific competitive commercial ELISA kits in 50 µL of lavage fluid. NAL was not concentrated for neuropeptide measurement. SP (Cayman Chemical Company, Ann Arbor, Michigan, USA) was calculated as pg/mL of lavage fluid (minimal detectable concentration, 7.8 pg/mL). NKA and VIP (Peninsula Laboratories, Inc., Belmont, California, USA) were calculated as ng/mL of lavage fluid (minimum detectable concentration for NKA, 0.06-0.08 ng/mL; and for VIP, 0.04-0.06 ng/mL).

For histamine analysis, the supernatants were assayed after protein precipitation with a volume of 2% HClO$_4$ by an automated fluorometric technique capable of detecting 0.5 ng/mL of histamine [20].

The total protein concentration was determined using a modified version of Lowry's assay (BCA Protein Assay Reagent, Pierce 23225, Thermo Fisher Scientific, Rockford, Illinois, USA, with a detection limit of 10 µg/mL) and the neuropeptide to total protein ratio was calculated.

Human serum albumin (HSA) in lavage samples was determined using ELISA as follows: an immunosorbent microtiter plate (NUNC, Wiesbaden, Germany) was coated with HSA (5 µg/mL) and incubated overnight at 4°C. Nonspecific binding sites were blocked with 1% bovine serum albumin incubated for 1 hour at room temperature and washed with TRIS buffer. Appropriate dilutions of the samples and HSA standard (Sigma Chemicals) were placed on a Greiner microtiter plate. Dilutions (1:100 000) of purified mouse antihuman serum albumin (ImmunoPure® Mouse Monoclonal Anti-human Serum Albumin Assay Reagent kit [detection limit, 10 µg/mL]; Pierce 37108X, Thermo Fisher Scientific) were added and incubated for another hour at room temperature. Then, 150 µL was transferred to the coated NUNC plate and incubated for 1 hour, before being washed 3 times with TRIS buffer and incubated with alkaline phosphatase–conjugated AffiniPure goat anti-mouse IgG (Dianova, Hamburg, Germany) at a 1:1000 dilution for 1 hour. The wells were then washed 4 times and the reaction was developed with p-nitrophenyl phosphate (Sigma Chemicals) and the absorbance read at 405 nM. The standard curve for HSA ranged from 9.75 ng/mL (detection limit) to 10 000 ng/mL.

Statistical Analysis

Results are presented as the mean (SEM). Clinical scores, nasal airflow, and the concentrations of the different mediators in the NAL fluids (expressed in pg/mL, ng/mL, or µg/mL) were compared using the Wilcoxon signed-rank test (pre/postchallenge and pre/post–fluticasone propionate). The Mann-Whitney test was used to compare data between groups (patients versus healthy controls).

A P value of <.05 was regarded as statistically significant. The Spearman correlation coefficient (r) was calculated to analyze possible correlations between 2 different parameters; a correlation was considered significant at P<.05.

Results

Clinical Data and Rhinomanometry

All patients completed the study with 2 allergen challenges; no adverse effects were reported. Two weeks' treatment with fluticasone propionate resulted in a significant reduction in nasal symptoms in the patients. The mean score for nasal blockage decreased from 2.7 to 1.0, for rhinorrhea from 2.5 to 0.45, for itching from 1.9 to 0, and for sneezing from 2.0 to 0. The total symptom score fell from 9.1 to 1.45 ($P<.001$) and the mean number of sneezes from 9 to 1. In the early phase (0-10 minutes), all 11 allergic participants had a pronounced clinical reaction after the first challenge (pretreatment), whereas controls showed no reaction. Rechallenge revealed that treatment with fluticasone propionate reduced the severity of all symptoms significantly ($P<.01$) (Figure 2).

Prechallenge nasal flow and resistance were similar in the patient and in the control group, and were not significantly affected by the challenge with diluent.

However, in the untreated patient group, there was a significant decrease in the mean nasal flow rate 10 minutes after allergen challenge from 369 cm³/s to 135 cm³/s ($P<.004$) (Figure 3) and a 3-fold increase in mean nasal airway resistance from 0.41 Pa/cm³/s to 1.38 Pa/cm³/s ($P<.05$). After 2 weeks of treatment with fluticasone propionate, the nasal challenge was

Figure 3. Effect of 2 weeks of treatment with fluticasone propionate on changes in nasal airflow at 150 Pa/cm³/sec during nasal challenge: baseline, 10 minutes after diluent challenge, and 10 minutes after allergen challenge. The flow values, expressed as means and differences in the allergic group before and after treatment with fluticasone propionate, were compared using the Wilcoxon test ($P<.001$) and between allergic patients and controls before treatment using the Mann-Whitney test ($P<.01$).
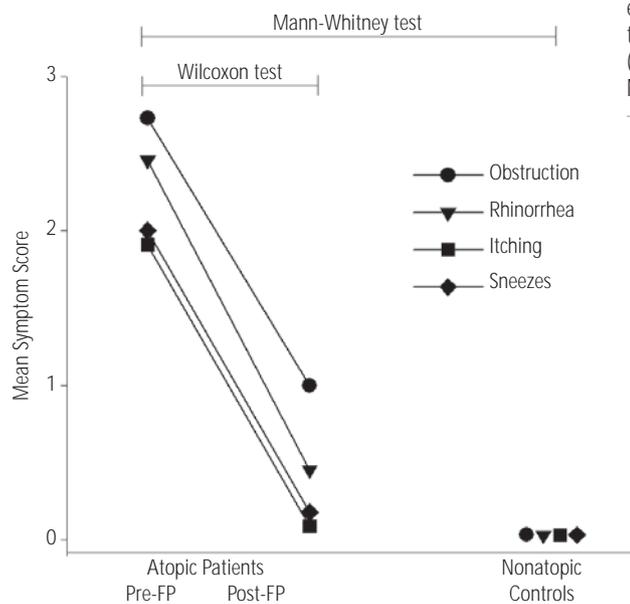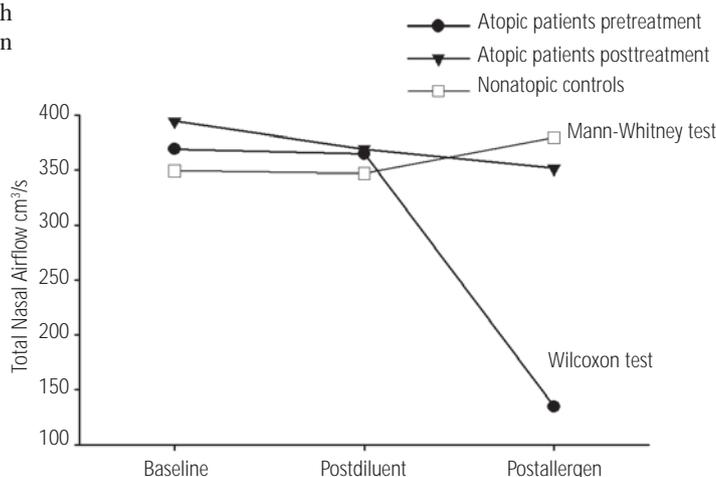

Figure 2. Significant improvement in clinical response after allergen challenge (10 minutes) following 2 weeks of treatment with fluticasone propionate (200 μg qd) in patients with persistent allergic rhinitis. The symptom score values, expressed as means and differences in the allergic group before and after treatment with fluticasone propionate, were compared using the Wilcoxon test ($P<.001$), and between allergic patients and controls, before treatment using the Mann-Whitney test ($P<.001$). FP indicates fluticasone propionate.

repeated. A significant change in the total symptom score was not observed in patients or in controls for nasal flow or nasal airway resistance.

Mediator Levels in Nasal Lavage Fluid

The baseline concentrations of SP, NKA, and VIP in the first nasal lavage (prewash) were similar in the allergic group and in the control group and there were no significant differences (Table 1). The diluent challenge, used as a control, did not induce a significant change in mediator concentrations in any participant. In the allergic group, the mean SP baseline NAL concentration was 29.6 (6.5) pg/mL. After allergen challenge, a significant increase was observed, with a maximum after 5 minutes of 135.3 (32) pg/mL ($P<.003$) compared to baseline and diluent challenge levels. This increase was still significant 10 and 20 minutes after allergen challenge. After treatment with fluticasone propionate, baseline SP levels were significantly lower ($P<.003$), and no increase was observed after rechallenge. In the control group, the mean

Table 1. Neuropeptide, Histamine, and Protein Concentration in Nasal Lavages Before and After Allergen Challenge[a]

	Patient	Control	Atopic Pretreatment	Atopic Posttreatment
SP, pg/mL	Baseline	23.10 (2.50)	33.00 (5.60)	26.50 (3.90)
	Diluent	17.20 (4.20)	29.60 (6.50)	28.90 (5.90)
	5 min PC	22.40 (3.30)[b]	135.30 (32.30)	41.60 (11.30)[e]
	10 min PC	16.70 (3.70)[c]	117.40 (30.90)	40.70 (11.60)[e]
	20 min PC	18.50 (3.40)[d]	79.90 (22.80)	29.30 (3.60)[f]
	30 min PC	19.90 (3.90)[d]	54.20 (13.70)	22.70 (2.50)[f]
NKA, ng/mL	Baseline	0.09 (0.03)	0.18 (0.02)	0.05 (0.01)
	Diluent	0.11 (0.04)	0.11 (0.03)	0.10 (0.04)
	5 min PC	0.14 (0.04)[b]	1.36 (0.40)	0.40 (0.15)[e]
	10 min PC	0.15 (0.03)[e]	1.40 (0.58)	0.38 (0.17)[e]
	20 min PC	0.15 (0.03)[d]	0.49 (0.21)	0.12 (0.03)[f]
	30 min PC	0.18 (0.04)	0.27 (0.08)	0.08 (0.02)[f]
VIP, ng/mL	Baseline	0.13 (0.04)	0.27 (0.09)	0.12 (0.04)
	Diluent	0.11 (0.03)	0.16 (0.06)	0.09 (0.07)
	5 min PC	0.13 (0.03)[b]	2.15 (0.48)	0.54 (0.20)[f]
	10 min PC	0.11 (0.02)[b]	2.35 (0.56)	0.51 (0.18)[e]
	20 min PC	0.13 (0.03)[d]	1.38 (0.44)	0.24 (0.08)[f]
	30 min PC	0.10 (0.03)[d]	0.64 (0.19)	0.25 (0.08)[f]
Histamine, ng/mL	Baseline	0.78 (0.07)[d]	1.30 (0.21)	0.65 (0.10)[c]
	Diluent	0.73 (0.12)	1.03 (0.19)	0.63 (0.09)
	5 min PC	0.71 (0.11)[c]	3.71 (0.69)	0.72 (0.11)[b]
	10 min PC	0.61 (0.09)[c]	4.26 (0.64)	0.65 (0.10)[b]
	20 min PC	0.53 (0.08)[d]	2.52 (0.58)	0.59 (0.07)[c]
	30 min PC	0.45 (0.06)[d]	1.46 (0.22)	0.47 (0.06)[c]
Protein, µg/mL	Baseline	29.16 (3.23)	39.63 (5.28)	35.44 (3.22)
	Diluent	30.19 (3.79)	37.96 (5.11)	32.14 (3.81)
	5 min PC	30.69 (3.84)[b]	150.66 (31.05)	48.61 (7.19)[e]
	10 min PC	24.21 (3.59)[c]	95.07 (17.10)	48.92 (7.30)[e]
	20 min PC	21.44 (3.97)[d]	72.06 (14.61)	32.96 (2.68)[f]
	30 min PC	24.09 (3.35)[d]	67.74 (16.09)	31.62 (4.46)[f]

Abbreviations: NKA, neurokinin, A; PC, postchallenge; SP, substance P; VIP, vasoactive intestinal peptide.
[a]Values are given as mean (SEM). Values are shown for baseline levels, 10 minutes after diluent (control challenge), and 5, 10, 20, and 30 minutes after challenge with allergen (*Dermatophagoides pteronyssinus* 100 000 AU/mL).
[b]$P<.001$ (Mann-Whitney test)
[c]$P<.01$ (Mann-Whitney test)
[d]$P<.05$ (Mann-Whitney test)
[e]$P<.01$ (Wilcoxon test)
[f]$P<.05$ (Wilcoxon test)

SP concentration was lower—23.1 (2.5) pg/mL—and did not change after challenge. Similar results were observed for total protein (Table 1). Levels of NKA, VIP, and histamine reached a maximum between 5 and 10 minutes after allergen challenge with significant elevations after 20 and 30 minutes (Table 1). Similar results were seen for albumin (HSA) in nasal lavage fluid, namely, a significant increase from baseline levels (5.3 [0.6] µg/mL) after allergen challenge, a maximum after 10 minutes (45 [5.3] µg/mL; $P<.01$), and no significant changes after treatment with fluticasone propionate (mean baseline levels, 1.3 [0.4] µg/mL vs mean concentration 10 minutes after allergen challenge 8.5 [0.7] µg/mL) or in the control group (1.2 [0.22] µg/mL vs 1.4 [0.25] µg/mL).

In order to determine whether the increased neuropeptide levels only reflected increased release of total protein, the ratio of neuropeptide (SP, NKA, VIP) to total protein was calculated for each lavage. The peak increase in SP, NKA, and VIP 5 and 10 minutes after allergen challenge occurred with the highest ratio of neuropeptide to total protein. Thus, the increased NP values represent a real increased release of these peptides in addition to the increased protein leakage. The results and the statistical evaluation are summarized in Table 2.

Table 2. Ratio of Substance P, Neurokinin A, and Vasoactive Intestinal Peptide to Total Protein in Nasal Lavage Fluids[a]

	Baseline		Five Minutes After Challenge		Ten Minutes After Challenge	
	Patients	Controls	Patients	Controls	Patients	Controls
SP/TP,	0.88	(0.8)	1.18	(0.9)	1.30	(1.57)
pg/mL/μg/mL	0.77	(0.15)	0.75	(0.32)	0.64	(0.34)
NKA/TP	2.68	(3.1)	16.3	(12.9)[b]	13.60	(10.7)[b]
pg/mL/μg/mL	5.84	(4.59)	5.75	(4.32)[c]	6.2	(4.22)[c]
VIP/TP,	6.79	(3.0)	2.48	(2.9)	23.60	(15.6)[b]
pg/mL/μg/mL	5.02	(4.14)	4.9	(4.32)	5.4	(3.4)[c]

Abbreviations: NKA, neurokinin A; SP, substance P; TP, total protein; VIP, vasoactive intestinal peptide.
[a]Values are given as mean (SEM).
[b]$P<.05$ (Wilcoxon test)
[c]$P<.05$ (Mann-Whitney test)

Correlations

We analyzed data from patients before and after treatment with fluticasone propionate to establish a correlation between symptom score, nasal flow rate, and levels of mediators in nasal lavage fluid. There was a good correlation between the symptom score and the NP levels 10 minutes after allergen challenge—SP (r=0.59, $P<.002$), NKA (r=0.54, $P<.0046$), VIP (r=0.65, $P<.005$)—and a negative correlation with the higher symptom score and lower nasal flow rate (r=–0.79, $P<.001$). There was also a significant correlation between changes in nasal flow rate before and after allergen challenge (Δ values from baseline and 10 minutes after allergen challenge) and the changes in NP levels before and after (10 minutes) allergen challenge, with higher NP levels resulting in lower nasal airflow: SP (r=–0.56, $P<.003$), VIP (r=–0.53, $P<.006$), and NKA (r=–0.36, $P<.05$).

Discussion

In the present study, we observed relevant quantities of the 3 neuropeptides in nasal lavage fluid in a group of patients suffering from persistent allergic rhinitis compared with a control group of healthy volunteers at baseline. In a second step, we investigated the effect of intranasal fluticasone propionate on clinical symptoms and NP levels in nasal lavage fluid in the study population; a postchallenge decrease was observed in SP, NKA, VIP, histamine release, HSA, and total protein in patients who had taken fluticasone propionate.

Inflammation in allergic rhinitis has been associated with several mediators, inflammatory cells, and stimulation of C fibers. Since release of mediators into the airway lumen plays an important role in the pathogenesis of airway disease, lavage-based techniques have been performed in patients with allergic rhinitis to study underlying mechanisms [21,22]. SP and NKA are present in afferent sensory neurons of the trigeminal nerves of human nasal mucosa, and VIP coexists with acetylcholine

in parasympathetic neurons. Different interactions have been described. A recently published study demonstrated that SP and VIP induce mast cell degranulation [23]. Histamine released from mast cells produces a number of symptoms, such as hypersecretion. It also depolarizes sensory nerves through its receptors, with consecutive release of neuropeptides. The depolarization wave on the surface of nerve cells is then centrally conveyed to the secretory-vasomotor and sneezing centers of the brain [8,24].

The present study demonstrates that topical fluticasone propionate has a significant effect on clinical reaction and symptoms and also on mediator release compared with pretreatment values. The ratio of neuropeptide to total protein in the nasal lavage fluid of patients with HDM allergy was significantly higher after allergen challenge than that of healthy controls or allergic patients treated with fluticasone propionate. Studies by Nieber et al [25], Mosiman et al [26], Walker et al [27], and Tønnesen et al [28] have also demonstrated a dose-dependent increase in SP and VIP in nasal lavage fluid and bronchoalveolar lavage after allergen provocation in allergic patients. Since VIP is composed of 28 amino acids that mainly act through the VPAC1 and VPAC2 receptors, one of its functions is induction of glandular secretion. In neurogenic inflammation it is more an inhibitory modulator of inflammation [3], whereas SP and NKA are promoters of neurogenic inflammation. Okamato et al [29] showed that SP upregulates mRNA for the proinflammatory cytokines IL-1β, IL-3, IL-5, IL-6, TNF-α, and INF-α, which could be an additional stimulus to allergic inflammation.

Different mechanisms could contribute to the decreased release of neuropeptide in nasal lavage fluid caused by fluticasone propionate in our study. Corticosteroids are able to upregulate the synthesis of neuropeptide-degrading enzymes [30] and downregulate tachykinin receptors and neuropeptide synthesis in neurons and in other immune cells [31], although they can also decrease type 2 helper T cell cytokine levels [32].

One study showed that fluticasone nasal spray reduces the nasal-ocular reflex, thus decreasing ocular symptoms and

revealing complex interactions in the process of neurogenic inflammation [33].

The present results demonstrate that persistent allergic rhinitis in humans is accompanied by elevated concentrations of SP, NKA, and VIP in NAL after allergen challenge. We also show that topical fluticasone is effective in inhibiting the nasal response to allergen challenge. This finding might contribute to a further understanding of neuronal and immunologic pathways in allergic rhinitis and airway inflammation.

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▌ **Dr Christoph Schäper**

Department of Internal Medicine B – Cardiology, Intensive Care, Pulmonary Medicine and Infectious Diseases, Universität Greifswald, Greifswald, Germany
Friedrich-Loeffler-Str. 23 a
17475 Greifswald
Germany
E-mail: christoph.schaeper@googlemail.com