

Effects of β -endorphin on nasal allergic inflammation

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Summary

Background β -endorphin is a derivative of pro-opiomelanocortin. Cells of the immune system can also synthesize and secrete β -endorphin. Its concentration is increased during the allergic reaction and during stress. Increased reactivity during psychological stress of allergic subjects is also well known.

Objective Is β -endorphin one physiological link between stress and an exacerbation of the allergic reaction?

Methods First, intranasal β -endorphin challenges with subsequent lavages to determine histamine and albumin levels and measurements of nasal flow and resistance in dose-response and time course experiments were performed. Secondly, we examined whether β -endorphin pre-treatment increased the antigen-induced release of histamine and albumin in nasal lavages and the clinical symptoms.

Results Exogenous β -endorphin (100 pM–10 μ M/mL) induced a dose-dependent increase in nasal symptoms in asymptomatic allergic subjects with rhinitis ($n=14$) as well as in non-allergic controls ($n=10$), but did not release any mediators into nasal secretion. However, comparing the antigen-evoked release of mediators into nasal secretions with that of a β -endorphin pre-treated antigen challenge we could note a significant enhancement of human serum albumin influx ($P<0.05$) and histamine liberation ($P<0.05$) 10 min after antigen challenge compared with the allergen challenge alone, with also a correlation with the more pronounced decrease in nasal flow ($P<0.05$).

Conclusion These results suggest that β -endorphin-induced increase in nasal congestion is mediated through direct neuroendocrine receptor activation independent of mast cell activation and that during the allergic reaction there is a β -endorphin/mast cell interaction that enhances the mediator response to nasal allergen challenge.

Keywords allergen challenge, β -endorphin, histamine, human serum albumin, nasal lavage

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Introduction

β -endorphin is an endogenous peptide opiate comprising 31 amino acids and is a derivative of pro-opiomelanocortin (POMC). Together with the other POMC peptides, in particular corticotrophin releasing factor (CRF), adrenocorticotrophin hormone (ACTH) and alpha-melanocyte stimulating hormone (α -MSH), β -endorphin is a powerful immunoregulator that has a direct regulatory effect on lymphoid cells, regulating immune reactions by the stimulation of immunoregulatory hormones (glucocorticoids) and also by acting on the central nervous system, which in turn generates immunoregulatory nerve impulses [1–3]. Peptidergic nerves are major regulators of the inflammatory response. There is evidence that after allergen-induced inflammation neuropeptide expression in afferent sensory nerves in guinea-pigs is increased [4], and it has been shown that nasal challenge of allergic humans with antigen

as well as with bradykinin can induce the release of the neuropeptide, substance P [5,6].

β -endorphin is synthesized mainly in the arcuate nucleus of the hypothalamus, and projects into several brain areas [7]. Circulating β -endorphin might derive either from the pituitary [7], or from a number of immune cells where it is synthesized. In recent studies it has been demonstrated that the cells of the immune system can also express the POMC gene and synthesize and secrete many POMC-derived peptides such as β -endorphin and ACTH [8–10]. Human T and B lymphocytes and monocytes are able upon appropriate stimulation to produce and release β -endorphin [10–12]. Furthermore, an increase of PBMC β -endorphin concentrations has been observed in conditions when immunosuppression and the Th2 cytokine pattern prevail, e.g. in cells obtained from HIV-positive subjects or after stress [13,14].

Current evidence suggests that neuroimmune mechanisms are essential in normal physiology and, on the other hand, abnormalities contributing to the aetiology of chronic inflammatory diseases, allergy, and asthma. Increased baseline levels of β -endorphin in bronchoalveolar lavage fluid of allergic subjects and a dose-dependent increase in β -endorphin levels following a segmental intrabronchial antigen challenge in

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allergic patients [5] suggest a neural activity in man. As the concentration of β -endorphin rises significantly during stress-induced situations in the plasma of humans [22,23], the observation of increased reactivity during psychological stress of allergic subjects supports the hypothesis that mast cell degranulation is modulated by neural activity. β -endorphin seems to be one physiological link between the psychological phenomenon of stress and the physical manifestation of the allergic response, for example an asthma attack. Indeed, we could show in earlier studies that the tachykinin substance P enhances the antigen-evoked mediator release to nasal allergen challenge [24].

We now demonstrate that intranasal administration of β -endorphin strengthens the allergic response, apparently by making the patient more sensitive to the allergen.

Materials and methods

Subjects

A total of 14 volunteers, with a history of seasonal or perennial allergic rhinitis and a positive skin prick test response to grass pollen or house-dust mite antigen and specific IgE levels with a CAP class >2 , were recruited (eight females, six males, age range 20–40 years). Ten healthy controls (five females, five males, age range 19–50 years) were also recruited. Subjects presenting with any evidence of respiratory tract infection or nasal

polyposis, were excluded. No subject was taking medication at the time of, or in the 2 weeks prior to, the study, nor had any subject received immunotherapy. The study was performed 3 months before the beginning of the pollen season. All subjects gave their informed consent. The study was approved by the local ethics committee at the Humboldt University Berlin.

Materials

β -endorphin (NOVA Biochem, Schwalbach, Germany) was prepared in 0.9% sterile saline with 0.25% human serum albumin, which also served as the control. It was applied to the nasal cavity using a mechanical nasal pump spray that delivered a volume of 0.1 mL per actuation. The device produces widespread distribution of the given fluid in the nasal cavity. Fresh solutions were made each day from stock solutions stored at -20°C , and were allowed to equilibrate with room temperature prior to administration.

The technique of nasal challenge with subsequent lavage has previously been described in detail [25,26]. Briefly the nasal lavage was carried out by instilling 5 mL sterile saline into each nostril, using a syringe with a nasal olive attached. Subjects were positioned with the head bent forward, to prevent loss of fluid into the nasopharynx. The lavage fluid was passed into the nasal cavity and back into the syringe after 10 s before being collected in polypropylene tubes and stored on ice. Recovery by this method was $89 \pm 10\%$.

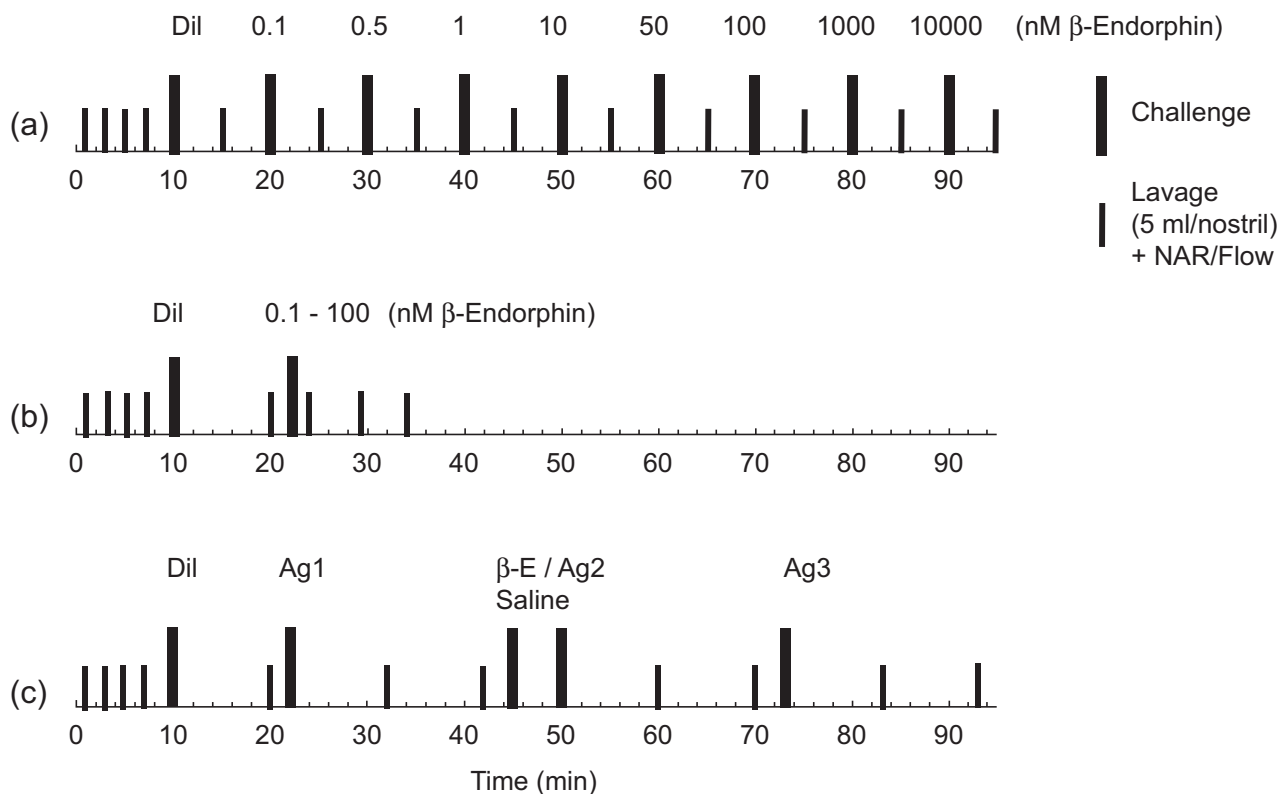


Fig. 1. (a) The experimental protocol of the first part of the study. Increasing dosages of β -endorphin were applied to the nasal mucosa of normal volunteers and patients with allergic rhinitis at 10-min intervals to determine dose-responses. Flow and resistance measurements were performed 5 min after each β -endorphin application. (b) Time-course experiments with 0.1 pM–100 nM β -endorphin with determinations of NAR and nasal flow (1, 2, 5 and 10 min later) and consecutive nasal lavages (2, 5, 10 min later, following 100 nM β -endorphin). (c) The experimental protocol of the third part of the study. The allergic patients underwent a sequence of three successive nasal allergen challenges (Ag 1–3), each followed by two nasal lavages at 10- and 20-min intervals. Each challenge with the antigen was separated by 35 min. Five minutes before the second nasal allergen challenge a β -endorphin (100 nm/mL β -endorphin in each nostril) or saline control was performed. Dil = diluent (albumine buffered saline).

The nasal flow decrease was determined by anterior rhinomanometry using a WinRhino PC-Rhinomanometer 600 (Allergopharma J. Ganzer, Reinbek, Germany). The rhinomanometry allows an objective and quantifiable statement to be made about the patency (resistance characteristics) of the nose. In physical terms, the resistance may be described by the air flow through the nose as a function of the inspiratory and expiratory pressure difference between the two choanae and the area in front of the nostrils. The rhinomanometer records the two quantities synchronously in physiological terms by measuring the subjects' spontaneous breathing. A positive nasal provocation will cause a reduction of flow at $P=150$ Pascal, by 40% or more, compared with the flow at $P=150$ Pascal of the diluent measurement.

For each determination of flow, triplicate measurements were made on each side of the nasal airway. Data are presented as the mean flow of both sides (cm^3/s).

Each patient and normal volunteer was provided with a symptom score card on which the nasal symptoms were recorded at the same time as the lavages following the β -endorphin challenge. The symptoms of nasal blockage, rhinorrhea and nasal itching were rated on a four-point scale with 0=no symptoms, 1=mild, 2=moderate and 3=severe symptoms. A composite nasal symptom score was then calculated from the individual symptoms as previously described [27].

Study design

In each protocol, four initial washes were performed to remove any pre-existing mediators, and the fourth wash retained and used as a baseline. The subjects were then challenged with saline

control (albumin buffered saline) and nasal lavages were performed 10 min after the diluent challenge.

In the first part of the study increasing doses of β -endorphin (100 pM–10 $\mu\text{M}/\text{mL}$) were applied topically to the nasal mucosa of 10 normal volunteers and 14 patients with allergic rhinitis at 10-min intervals to determine dose-responses. Nasal lavages and flow and resistance measurements were performed 5 min after each β -endorphin application (Fig. 1a). In the absence of β -endorphin challenge, repetitive saline lavages did not induce changes in nasal flow. Furthermore, sequential exposures at 10-min intervals with the same dose of β -endorphin revealed similar decreases of nasal flow, which excludes a cumulative effect due to carryover from the previous challenge. The effect of β -endorphin was not influenced by an oral pre-medication with 1.34 mg clemastinhydrogenfumarat ($n=7$) (Tavegil, Novartis Consumer Health; Munich, Germany) 60 min prior to the challenge.

In the second part, time-course experiments with increasing doses of β -endorphin (100 pM–10 $\mu\text{M}/\text{mL}$) were carried out with consecutive determination of nasal airway resistance (NAR) and nasal flow (1, 2, 5 and 10 min later). In addition, nasal lavages (2, 5 and 10 min later) were performed after 100 nM β -endorphin application (Fig. 1b). The lavage fluid was processed for the chemical analysis of histamine, tryptase and albumin as described below.

In the third part of the study we examined whether β -endorphin increased antigen-induced release of histamine and albumin. The allergic subjects underwent a sequence of three successive nasal allergen challenges, each followed by two nasal lavages at 10- and 20-min intervals and determination of

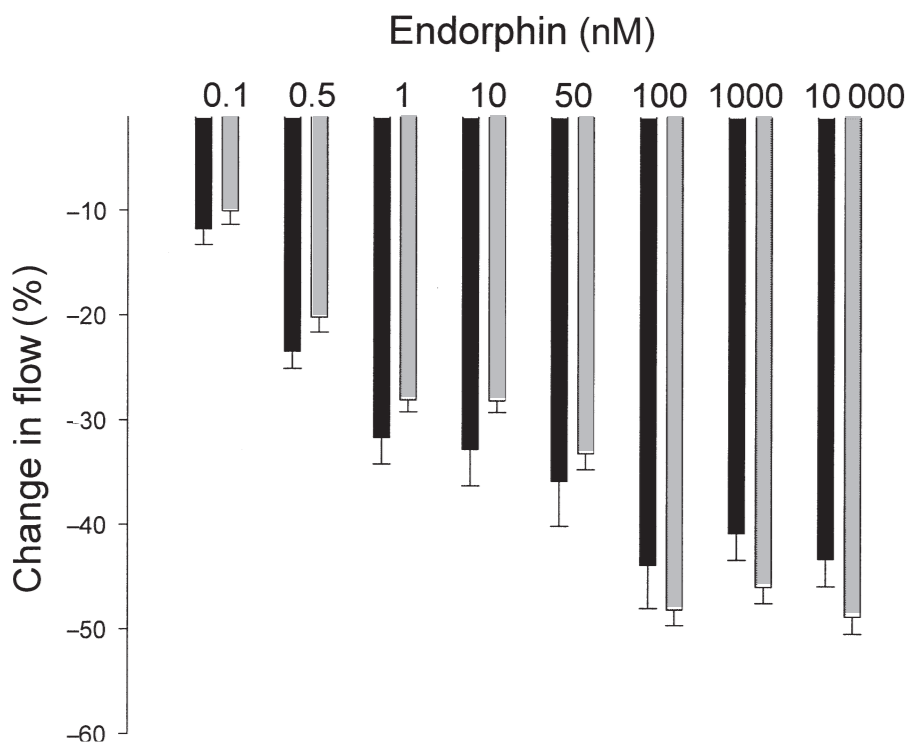


Fig. 2. Endonasal challenges of both groups with increasing dosages of β -endorphin (100 pM–10 $\mu\text{M}/\text{mL}$) induced dose-dependent decrease in nasal flow representing mainly symptoms of nasal congestion. The decrease in nasal flow was already significant after administration of 0.5 nM β -endorphin ($P < 0.05$) and reached a level of $P < 0.001$ with the higher doses of β -endorphin used (baseline vs. 100 nM β -endorphin challenge). Black bars = 13 allergic patients without symptoms, grey bars = 10 controls.

nasal flow and NAR. Each challenge with the grass pollen or dust mite allergen (5000 SBE/mL of a commercial preparation, Allergopharma J. Ganzer) was separated by 35 min, according to the experiences of earlier studies [5,24–26]. Five minutes prior to the second nasal allergen challenge subjects received a nasal spray of β -endorphin (100 nM β -endorphin in each nostril) (Fig. 1c). With a minimum time interval of 4 weeks between both experiments these allergic subjects underwent another sequence of three successive nasal allergen challenges, each followed by two nasal lavages at 10- and 20-min intervals and determination of nasal flow and NAR, but 5 min before the second nasal allergen challenge they received a nasal spray of saline control (albumin buffered saline).

Biochemical analysis

Histamine was measured as previously described [26]. In brief, 0.8-mL aliquots for histamine analysis were mixed with 0.2 mL of 8% HClO₄, centrifuged and the supernatants were assayed by an automated fluorometric technique capable of detecting 0.5 ng/mL of histamine [28].

Tryptase in nasal lavage fluid was analysed by using the Pharmacia UniCap system (Pharmacia Diagnostics, Freiburg, Germany). A monoclonal antitryptase antibody is used for the detection of tryptase in this system. The samples were assayed according to the instructions of the manufacturers. The concentration of tryptase is expressed as nanograms per millilitres, and the lower limit of detection was 1 ng/mL.

Recovery experiments and tests of samples in various dilutions confirmed the absence of interference in nasal lavage fluid. β -endorphin (final concentration, 10 μ M/mL) did not interfere with the measurement of tryptase in the lavage fluid samples.

Human serum albumin (HSA) in the lavage samples was determined by using an ELISA as follows: an immunosorbant microtitre plate (NUNC, Wiesbaden, Germany) was coated with HSA (5 μ g/mL) and incubated overnight at 4°C. Non-specific binding sites were blocked with 1% BSA, incubated for 1 h at room temperature and washed with TRIS buffer. Appropriate dilutions of the samples and HSA standard (Sigma Chemicals, Munich, Germany) were placed on a Greiner microtitreplate; 1–100 000 dilutions of purified mouse anti-HSA (Pierce, Germany) were added and incubated for another hour at 37°C. Then 150 μ L were transferred to the coated NUNC plate and incubated for 1 h, then washed three times with TRIS buffer and incubated with alkaline phosphatase-conjugated AffiniPure goat antimouse IgG (Dianova, Hamburg, Germany) 1–1000 dilution for 1 h. The wells were

then washed four times and the reaction was developed with p-nitrophenyl phosphate (Sigma Chemicals) and the absorbance was read at 405 nm. The assay standard curve for HSA ranged from 9.76 (minimum detectable dosage) to 5000 ng/mL. All biochemical measurements carried out in a blinded fashion.

Statistical analysis

Statistical analysis was performed on an IBM compatible personal computer using the software STATGRAPHICS (STSC Inc., Rockville, MD, USA). The dimensions of the nasal airway vary between subjects and also within subjects from day to day, and so the data have been normalized by expressing changes in nasal flow as a percentage of the baseline value (control measurements following diluent). In all studies, the comparisons were made between active treatment and saline control. Independent samples (allergic subjects/controls) were compared using the Mann–Whitney *U*-test. Differences between dependent samples (pre/post- β -endorphin) were tested for significance with the Wilcoxon matched pairs signed rank test, and values of $P < 0.05$ were taken as significant.

Results

Pre-challenge nasal flow and resistance were similar in the controls and in the patients suffering from allergic rhinitis. Nasal flow and resistance were not significantly affected by the saline challenges. Endonasal challenges with increasing dosages of β -endorphin (100 pM–100 μ M/mL) caused a dose-dependent decrease in nasal flow from baseline 5 min after challenge ($P < 0.05$ for ≤ 500 pM) and were also similar in both groups (Fig. 2). Furthermore, the quantitative response to β -endorphin challenge was identical in the presence and absence of a potent H₁-antagonist. Clinical symptoms were nasal congestion parallel to nasal flow changes and, to a very low extent, nasal itching but no hypersecretion (Table 1). Systemic reactions or sensations were not observed in any case, even with the highest challenge dose.

The time-course experiments with changes of nasal flow from baseline following β -endorphin challenges are shown in Fig. 3, upper panel. These findings denote clearly increased nasal responsiveness, mediated by increased dosages. The highest changes in nasal flow were already seen 1–2 min following β -endorphin challenge for all dosages used. The lower the concentration of β -endorphin used, the earlier these changes in flow diminished.

Table 1. The composite nasal symptom scores (mean $\pm$ SEM), calculated from the individual symptoms (nasal blockage, rhinorrhea and nasal itching) in 10 control and 13 allergic individuals 5 min after 0.1–10 000 nM β -endorphin challenge

Dose (nM β -endorphin)	Controls	Allergic subjects
0.1	0.9 $\pm$ 0.27 1.3 $\pm$ 0.26	0.9 $\pm$ 0.18 1.2 $\pm$ 0.12
1	2.1 $\pm$ 0.23	2.2 $\pm$ 0.15
10	2.9 $\pm$ 0.18	2.8 $\pm$ 0.20
50	3.5 $\pm$ 0.22	3.0 $\pm$ 0.16
100	4.7 $\pm$ 0.21	3.8 $\pm$ 0.19
1000	4.9 $\pm$ 0.23	4.1 $\pm$ 0.18
10000	4.7 $\pm$ 0.15	3.9 $\pm$ 0.14

The observed differences between controls and allergic subjects at each dosage are not significant.

In all subjects, β -endorphin challenges did not result in an increase in mediators measured (Fig. 3, lower panel, shows the result for albumin and histamine following 100nM β -endorphin). Especially the levels of histamine in the lavage fluid did not show any significant changes over baseline level, and the levels of tryptase were always under the minimum detectable dosage of the assay, indicating that changes in nasal flow were not mediated by mast cell degranulation or influx of plasma constituents. These data indicate that β -endorphin causes nasal obstruction without histamine release or vascular leak.

Antigen provocation resulted in a decrease in percentage of nasal flow 10 and 20min later ($P < 0.001$) (Fig. 4, upper panel). Antigen challenge caused a significant increase in the histamine ($P < 0.001$) and albumin levels ($P < 0.05$) in the nasal lavage

fluids, both 10 and 20min later, compared with diluent control, as shown in Figs 5 and 6. Following pre-treatment with saline, there were no differences in the nasal flow measured and the mediator profile of the lavage samples taken after the second/following antigen provocation (Figs 4–6, upper panel). However, provocation with β -endorphin 5min before the second antigen challenges induced a significant increase in histamine ($P < 0.05$) and albumin ($P < 0.05$) in nasal lavage fluid, compared with the first antigen challenge (Figs 5 and 6, lower panel). The size of the effects on albumin and histamine is a twofold change/increase compared with antigen alone. All allergic patients noted nasal blockage, rhinorrhoea and sneezes but no systemic reactions during the repeated antigen challenge procedures. The symptoms did not differ following sequential antigen challenges (three challenges with saline pre-treatment

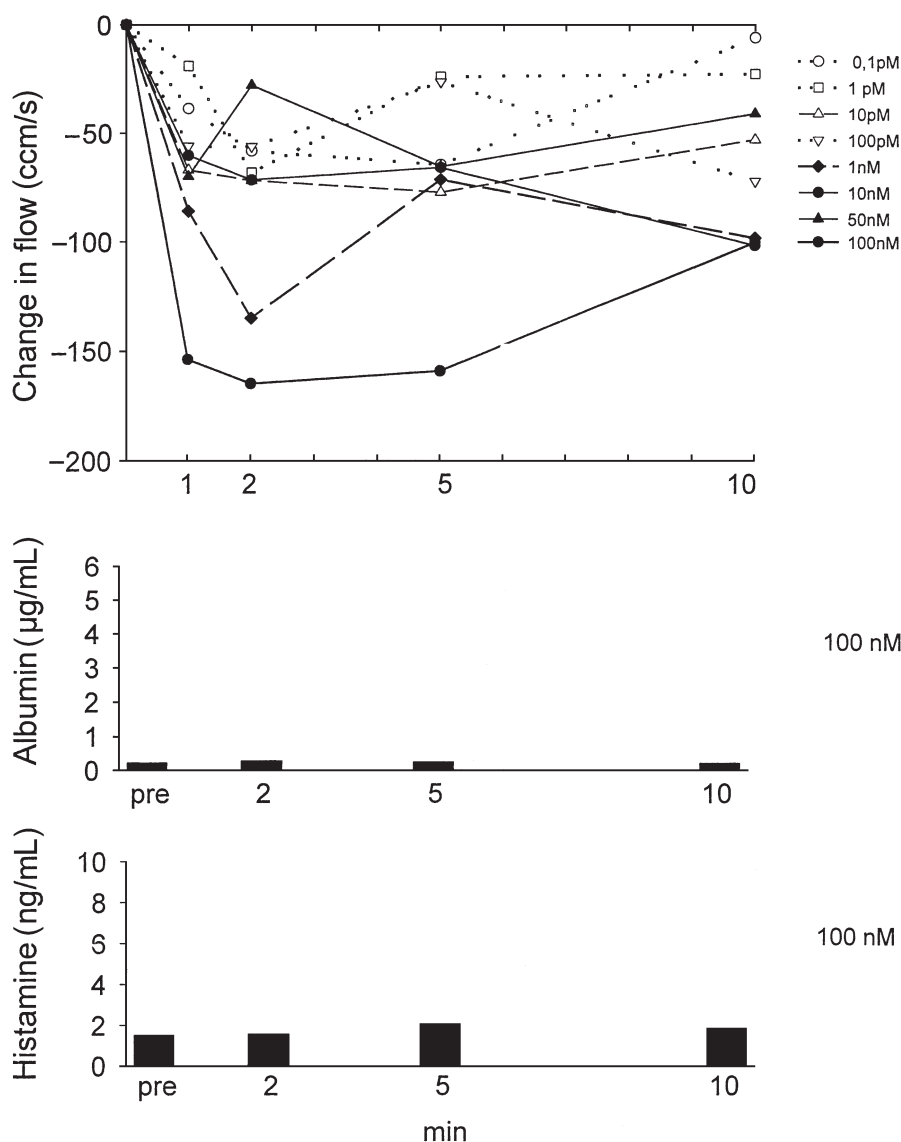


Fig. 3. The time-course experiments showing the changes between diluent- and β -endorphin-induced nasal flow (upper panel). The highest changes in flow were seen already 1–2min following challenge for all dosages of β -endorphin. The lower the concentration of β -endorphin used, the earlier these changes in flow diminished. The lower panel shows the results for histamine and albumin following 100nM β -endorphin: there is no significant change in the levels of these markers in the nasal lavage fluid and also no significant difference between normal subjects and allergic subjects in all experiments. The values are given as means. $n = 13$ (six controls and seven allergic patients without symptoms).

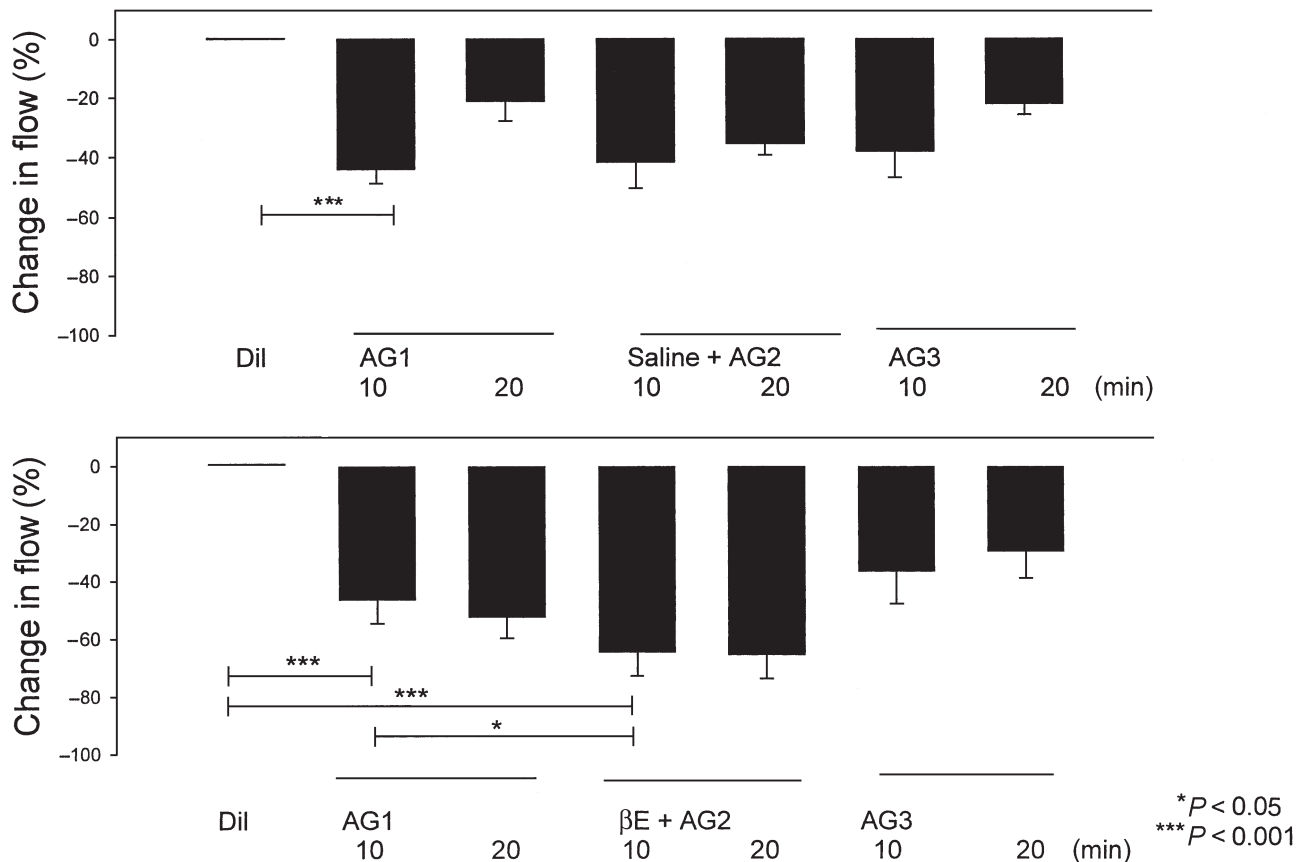


Fig. 4. The nasal flow induced by appropriate antigen challenge with or without pre-treatment with β -endorphin 5 min before the second antigen challenge. Data are means $\pm$ SEM from 14 asymptomatic allergic patients. Significant decrease in nasal obstruction for the β -endorphin pre-treatment shown, compared with antigen alone.

before the second challenge). The second β -endorphin pre-treated antigen challenge resulted in a significant increase of symptoms (not shown). Parallel to the symptom and mediator responses a more pronounced nasal airway flow reduction was seen in the β -endorphin pre-treated challenges ($P < 0.05$) (see Fig. 4, lower panel).

Discussion

Neurogenic inflammation plays a role in pathological conditions that include allergy, rhinitis, arthritis and gastrointestinal hypersensitivity [29–33]. Nasal symptoms have been attributed to the sympathetic and parasympathetic nervous system and the non-adrenergic non-cholinergic nervous system [34–36]. The distribution of peptidergic nerve fibres along gland acini and blood vessel walls strongly suggests their regulatory role in the secretory and vascular function of the nasal mucosa [37].

Hyper-responsiveness by neuronal stimulation may manifest as increased activity of the afferent, efferent, or even the elements of a central, reflex. The activation of peptidergic nerve fibres with local release of neuropeptides, demonstrated in nasal mucosa of rodents [38,39] and humans [6], when epithelial mucosa injury or mast cell degranulation occurs, and the responsiveness of the nasal mucosa to neuronal stimulation in

allergic subjects [24] reflects the direct secretagogue effect of neuropeptides released as part of an axonal reflex.

Bradykinin, neurotensin, VIP, acetylcholine and noradrenaline [40–43] have all been demonstrated to induce or enhance mast cell histamine release. However, mast cell production of certain cytokines can occur independently of the release of other mediators, e.g. histamine. Substance P has been shown to be capable of inducing an immediate release of TNF- α from rat mast cells at concentrations below those required to induce histamine release [44]. We have recently demonstrated that exogenous substance P induces nasal obstruction in a dose-dependent manner in normal subjects and patients with allergic rhinitis without classical mast cell degranulation [24]. The latter observation could also be explained by mast cell production of pro-inflammatory cytokines, which remains to be explored.

Similarly, we now have shown that exogenous β -endorphin induces nasal obstruction in a dose-dependent manner in both normal volunteers and patients with allergic rhinitis under conditions that do not induce histamine release or leakage.

One possible explanation could be that β -endorphin induces an unknown cytokine production by mast cells. As the only symptom was nasal blockage, it could be that the primary effect of β -endorphin was mediated through a direct neurokinin and/or other neuropeptide receptor activation.

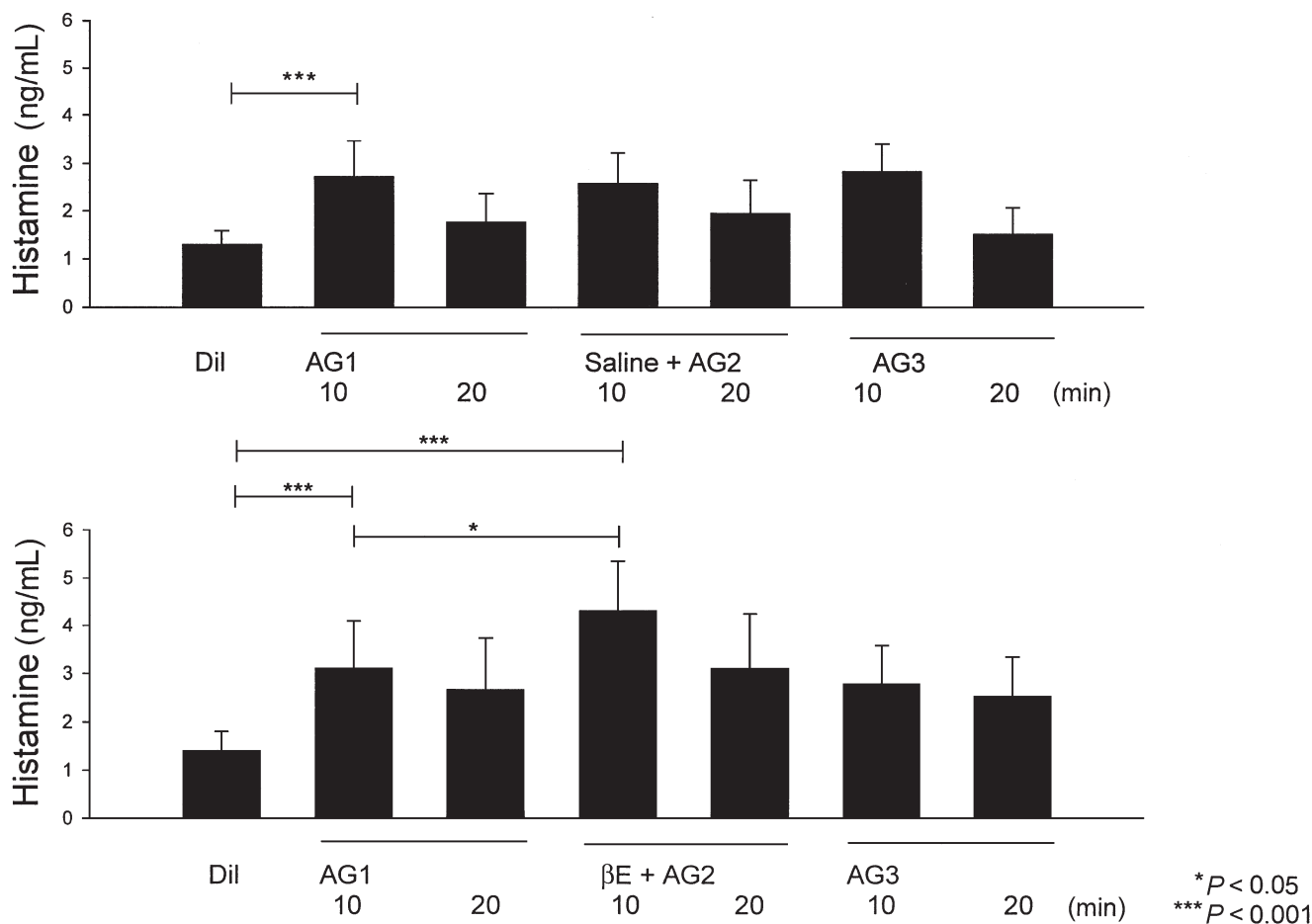


Fig. 5. Effect of successive nasal antigen challenge (Ag1 to Ag3), with or without pre-treatment with β -endorphin, 100nM, prior to the second antigen challenge, on the concentrations of histamine in the nasal lavage fluid. Data are means $\pm$ SEM from 14 asymptomatic allergic patients. β -endorphin given with allergen caused a twofold increase in histamine compared with allergen alone. The differences between the histamine levels of the allergen challenge procedures without pre-treatment of β -endorphin are not significant.

There is also evidence for nerve-mast cell interaction modulating the mast cell function. Masini et al. [45] demonstrated mast cell activation following stimulation of the viridan nerve in patients with chronic hypertrophic non-allergic rhinitis, and in the study mentioned above we have shown that substance P enhances the antigen-dependent histamine release from human nasal mucosa [24].

In asthma the role of psychological factors in disease exacerbation has been extensively reviewed [46,47]. However, there are few experimental data that directly implicate a neuroimmune/mast cell interaction or an interaction of elements of a central reflex with mast cells as a mechanism of disease modulation in man.

Higher baseline levels of β -endorphin in asthmatic patients compared with normal subjects, the demonstrated release of β -endorphin to local antigen challenge [6], and their production by cells of the immune system such as B and T lymphocytes and monocytes [10–12], suggest such a modulation, e.g. a mast cell interaction.

Although we could not demonstrate a β -endorphin-induced mediator secretion in the nose, we could provide direct evidence for this β -endorphin-mast cell interaction by a significant

increase of histamine concentrations in the lavage. As demonstrated in the present study, pre-treatment of the nasal mucosa with β -endorphin leads to an augmentation of the IgE-mediated mediator release.

Together with the observation that β -endorphin rises significantly during stress-induced situations [22,23], β -endorphin seems to be one physiological link between the psychological phenomenon of stress and the physical manifestation of an enhanced allergic response. These observations provide direct evidence that the immune system interacts with the neuroendocrine system. We believe that the central nervous system is the highest immuno-regulatory organ controlling immune and inflammatory reactions, with the aid of classical hormones, neurotransmitters and neuropeptides. The psychological stress of allergic subjects, combined with increased reactivity of these patients, possibly leads to a hyperactivity of the immune system.

In summary, we have shown that exogenously applied β -endorphin induces nasal congestion in allergic rhinitis and control subjects, without a β -endorphin-induced mediator release. However, in the atopic group we were able to demonstrate a β -endorphin-mast cell interaction enhancing the allergic inflammatory response.

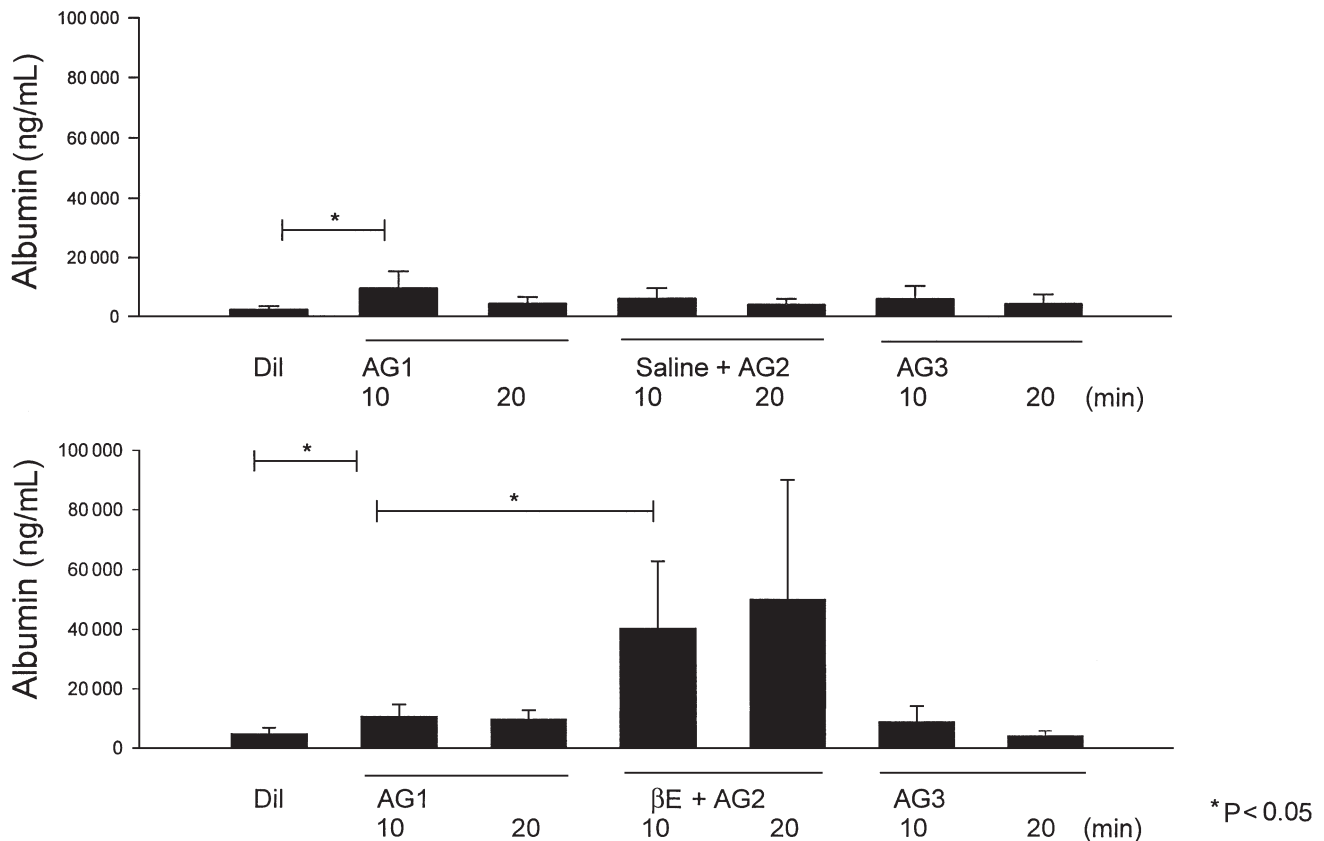


Fig. 6. Effect of successive nasal antigen challenge (Ag1 to Ag3), with or without pre-treatment with β -endorphin, 100nM, prior to the second antigen challenge, on the concentrations of albumin in the nasal lavage fluid. Data are means $\pm$ SEM from 14 asymptomatic allergic patients. β -endorphin given with allergen caused a large change in albumin, compared with allergen alone. The differences between albumin levels of the allergen challenge procedures without pre-treatment of β -endorphin are not significant.

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