

Nasal allergen deposition leads to conjunctival mast cell degranulation in allergic rhinoconjunctivitis

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ABSTRACT

Background: The naso-ocular interaction in allergic rhinoconjunctivitis is well recognized from epidemiological, clinical, and experimental observations. The precise mechanisms remain incompletely understood. A new mouse model of allergic rhinoconjunctivitis was used to investigate the contribution of mast cells and trigeminal ganglia activation to conjunctival (conj.) inflammation after nasal allergen provocation.

Methods: Sensitized mice were exposed to ovalbumin (OVA) via the nose and/or conjunctiva, and conj. homogenates were analyzed for histamine and substance P (using ELISA) and by eosinophil peroxidase (EPO) and beta-hexosaminidase assays. The conj. effects of nasal allergen deposition were compared with those induced by the mast cell activator C48/80 and with pretreatment of the mast cell stabilizer ketotifen or the transient receptor potential channel receptor (TRP) agonist capsaicin. Protachykinin 1 (TAC1) expression was quantified in the trigeminal ganglia using real time polymerase chain reaction.

Results: At 1 hour after nasal application of OVA, increased conj. levels of beta-hexosaminidase (0.68 ± 0.03 nm versus 0.56 ± 0.02 nm; $p = 0.02$), histamine (751.1 ± 52.17 ng/mL versus 546.3 ± 76.91 ng/mL; $p = 0.05$), and EPO (0.66 ± 0.09 nm versus 0.37 ± 0.03 nm; $p = 0.02$) were detected compared with saline. Higher levels of TAC1 expression were found in the trigeminal ganglia at 24 hours after OVA application (1326 ± 255 versus 687.5 ± 90.77 TAC1/beta-actin; $p = 0.04$). Nasal challenge with C48/80 increased substance P and beta-hexosaminidase levels in the conjunctiva, as well as TAC1 expression. Pretreatment with ketotifen resulted in lower levels of substance P as well as TAC1 expression. Destruction of sensory nerves in the nose by capsaicin reduced the OVA-induced conj. levels of substance P, histamine, and beta-hexosaminidase.

Conclusion: Nasal allergen deposition in sensitized mice induced trigeminal TAC1 expression and conj. mast cell degranulation. These data represent a significant step forward in understanding the close interaction between nasal and conj. inflammation in allergy.

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Allergic rhinitis (AR) and conjunctivitis often coexist in patients with pollen-induced allergic rhinoconjunctivitis, as highlighted by several epidemiological and experimental studies. Up to 70% of AR patients suffer from red, itchy, or watery eyes, with 15% of AR patients stating that their conjunctival (conj.) symptoms are at least as severe as their nasal symptoms.¹ Only recently, more insight was obtained in the mechanisms underlying the naso-ocular interaction in AR patients. We have reported conj. hyperemia, itching, and tearing in grass pollen AR patients after a selective nasal grass pollen provocation.² Mediators such as histamine and substance P are clearly involved, because their levels in tear fluid correlate with conj. symptoms and inversely correlate with nasal flow after a grass pollen provocation.^{2,3} Baroody *et al.* show that unilateral nasal allergen provocation gives rise to bilateral ocular itch and lacrimation.³ Alternatively, nasal filters in grass pollen-allergic individuals do not only prevent the induction of nasal symptoms but also conj. symptoms induced by natural pollen exposure.⁴ In line with the latter observation, nasal anti-inflammatory treatment reduces conj. symptoms in AR.^{3,5–10} Moreover, nasal treatment with fluticasone furoate reduces levels of proinflammatory mediators in the tear fluid, such as substance P and histamine.¹¹ Taken

together, the available data point toward an important role of nasal inflammation in the conj. immune homeostasis in AR patients. It is hypothesized that mast cell mediators, such as histamine, activate sensory neurons in the nasal mucosa,^{3,12} giving rise to the activation of the trigeminal ganglia beneath the brain stem. Activation of trigeminal ganglia may then lead to signals projecting to the conj. surface with the release of neuromediators. Activated neurons in the trigeminal ganglion synthesize neuromediators. Expression of the precursor gene for substance P and neurokinin A, protachykinin 1 (TAC1), is up-regulated in a mouse model of nasal inflammation caused by toluene diisocyanate.¹³ In asthma, studies performed in guinea pigs show increased levels of substance P, neurokinin A, and calcitonin gene-related peptide in the lung tissue accompanied with an elevated expression of TAC1 in the nodose ganglia, indicating that a *de novo* synthesis of neuromediators can be responsible for the late-phase airway hyperreactivity found in these guinea pigs.¹⁴ Moreover, polymorphisms in genes encoding for neuromediators such as TAC1 and TAC2 can be associated with a higher susceptibility for asthma.¹⁵

Mice with experimental airway allergy after sensitization and exposure to ovalbumin (OVA) present with most features of AR, such as circulating OVA-specific IgE and eosinophils releasing proinflammatory mediators such as eosinophil peroxidase (EPO) and cytokines.¹⁶ Murine models of allergic conjunctivitis elicited by topical allergen administration after systemic sensitization have mainly dealt with the role of Th2 cells and costimulatory molecules.^{17,18} Here, we developed a novel mouse model of allergic rhinoconjunctivitis that allowed us to study the conj. effects of nasal allergen provocation and to compare the effects of nasal versus conj. allergen challenge. The use of the mast cell activator compound 48/80 (C48/80), mast cell stabilizer, and histamine receptor 1 antagonist ketotifen allowed us to study the contribution and degranulation of mast cells by measuring the granular enzyme β -hexosaminidase. Capsaicin, which is an agonist of transient receptor potential cation channel receptor 1 (TRPV1) and is known to destroy sensory nerve endings,¹⁹ allowed us to study the involvement of nasal sensory nerves on the effects of nasal allergen provocation on the activation of trigeminal ganglia and conj. allergic inflammation.

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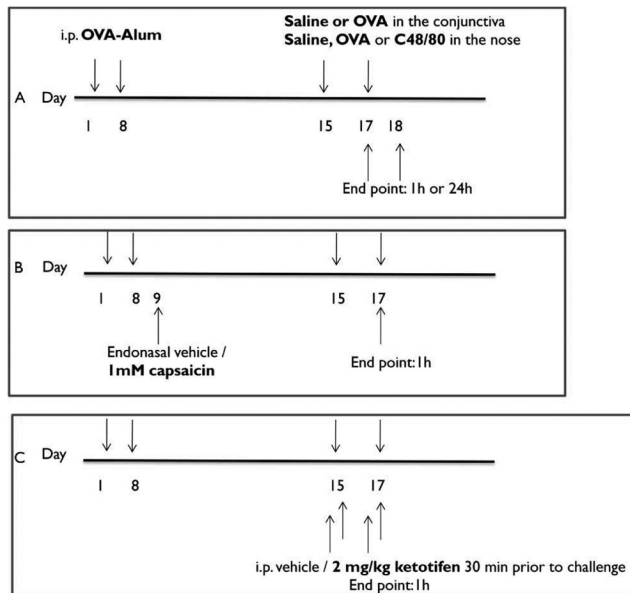


Figure 1. Study design with the different (A) experimental protocols and (B and C) treatment schedules.

MATERIALS AND METHODS

Mice

Male BALB/c mice 6–8 weeks of age were obtained from Harlan (Horst, the Netherlands) and were kept under conventional pathogen-free conditions. Experimental procedures were approved by the Catholic University of Leuven Ethical Committee. Mice were sensitized by an intraperitoneal (i.p.) injection of 320 μ g of OVA (Sigma-Aldrich, St. Louis, MO) with 1% aluminum hydroxide (Imject Alum; Thermo Scientific, Rockford, IL) on days 1 and 8. Mice were challenged on days 15 and 17 by dropping either 5 μ L of saline or 500 μ g of OVA in saline on the conjunctiva of both eyes and/or 50 μ L of saline or 500 μ g of OVA in the nose (endonasal [e.n.] challenge; Fig. 1; see supplemental data). All mice were conscious and fixed appropriately during the challenges to prevent mice from rubbing the solution from nose to eyes and *vice versa*. Mice were killed either at 1 or at 24 hours after the last challenge with an i.p. injection of 100 μ L of pentobarbital (Nembutal; Ceva, Bruxelles, Belgium). Bulbar and palpebral conjunctivae of both eyes were isolated microscopically. Homogenates were made of the conjunctivas using 350 μ L of PBS (Lonza, Verviers, Belgium) with 5% bovine serum albumin (Sigma-Aldrich). Levels of substance P (Cayman Chemical Co., Ann Arbor, MI) and histamine levels (Labor Diagnostika Nord, Nordhorn, Germany) were measured in the supernatants of these homogenates and were performed by means of commercially available ELISA kits.

Levels of β -hexosaminidase in the conj. homogenates were quantified by means of hydrolysis of 1 mmol/L of *p*-nitrophenyl *N*-acetyl- β -D glucosamide (Sigma-Aldrich) in 0.1 mol/L of sodium citrate buffer (pH 4.5) for 90 minutes at 37°C in the dark. The absorbance was read at 400 nm.

Real-Time Polymerase Chain Reaction

Total trigeminal ganglia and nasal mucosa were isolated and snap frozen until further analysis. Total mRNA was extracted from these tissues using RNeasy Mini Kit (74106; Qiagen, Germantown, MD). The obtained RNA was reverse transcribed using 200 U of Superscript II RT at 42°C for 60 minutes (18064014; Invitrogen, Gent, Belgium). Real-time polymerase chain reaction (PCR) was performed for TAC1 (forward [FW] primer, gaccgcaaatcgacatga; reverse [RV] primer,

ttggcatcgatttctctgc; and TaqMan probe, atcctcgtggccgtggcggt), TAC2 (FW primer, gtgggaccaaaaggagacatca; RV primer, gtcgggtggagtgctgtggt; and TaqMan probe, ttccacaga aacgtgacatgcacgact), and ubiquitin carboxy-terminal hydrolase L1 (UCHL1), which is the gene encoding for protein gene product 9.5 (PGP9.5; FW primer, gagctc-gatgggcgaatg; RV primer, cgctcagtgaattctctgcaga; and TaqMan probe, cctttccagtgaacctggtggccg) in the CFX Connect device from Biorad (Mississauga, Hercules, Canada). Mouse β -actin (FW primer, agagggaaatcgtgctgac; RV primer, caatagtgtgacacgtggcgt; and TaqMan probe, cactgcccatctcttctctcc) was used as a housekeeping gene. PCR amplification for each sample was performed using the following conditions: 10 minutes at 95°C, followed by a total of 40 two-temperature cycles (10 seconds at 95°C and 1 minute at 60°C). Primers and probes were designed using PrimerExpress software (Applied Biosystems, Foster City, CA).

Nasal Mast Cell Activation by C48/80

Sensitized mice were e.n. challenged with 60 or 120 μ g of C48/80, a mast cell activator (C2313; Sigma, St. Louis, MO), or saline, together with conj. saline on days 15 and 17 (Fig. 1 A).

Nasal Mast Cell Stabilization by Ketotifen

The mast cell stabilizer ketotifen (Sigma Chemical Co., St. Louis, MO) was dissolved in 200 μ L of 0.5% methylcellulose solution in dH₂O and administrated i.p. at 2 mg/kg, 30 minutes before e.n. challenge with OVA and conj. challenge with saline on days 15 and 17. Control mice received an i.p. injection of 200- μ L vehicle (0.5% methylcellulose in dH₂O; Fig. 1 C).

Destruction of the Nasal Afferent Nerve Endings by Capsaicin

Sensitized mice were given 1 mM of capsaicin (*N*-vanillylnonanamide; Sigma) or vehicle (solution of 96% alcohol, sodium dihydrogen phosphate, sodium chloride, disodium hydrogen phosphate, and H₂O) e.n. on day 9 (Fig. 1 B). To confirm the destruction of the sensory nerves in the nose, expression levels of UCHL1, which is the gene encoding for PGP9.5, a pan-neuronal marker, were measured by means of reverse-transcription PCR (PCR).

Statistical Analyses

A nonparametric Mann-Whitney test was used to test differences between groups. A difference was considered significant with a value of $p < 0.05$. Data are expressed as mean $\pm$ SEM.

RESULTS

Nasal Allergen Challenges Increased conj. Levels of β -Hexosaminidase, Histamine, and Substance P

Sensitized mice were challenged e.n. and in the conjunctiva with either saline or OVA. One hour after e.n. OVA challenge, increased levels of β -hexosaminidase were found in conj. homogenates (Fig. 2 A). In parallel, histamine and substance P levels showed similar trends with increased levels in mice challenged with e.n. OVA compared with saline (Fig. 2, B and C). Direct exposure of the conjunctiva to OVA also induced higher levels of conj. β -hexosaminidase (0.83 ± 0.02 nm versus 0.68 ± 0.03 nm; $p = 0.0087$, Fig. 2 A) and substance P (14.63 ± 1.47 pg/mL versus 10.27 ± 0.34 pg/mL; $p = 0.02$; Fig. 2 C).

Twenty-four hours after challenge, conj. levels of β -hexosaminidase and substance P were still increased in mice e.n. challenged with OVA compared with e.n. saline. However, histamine levels showed no differences in conj. homogenates in all groups at 24 hours after challenge (data not shown).

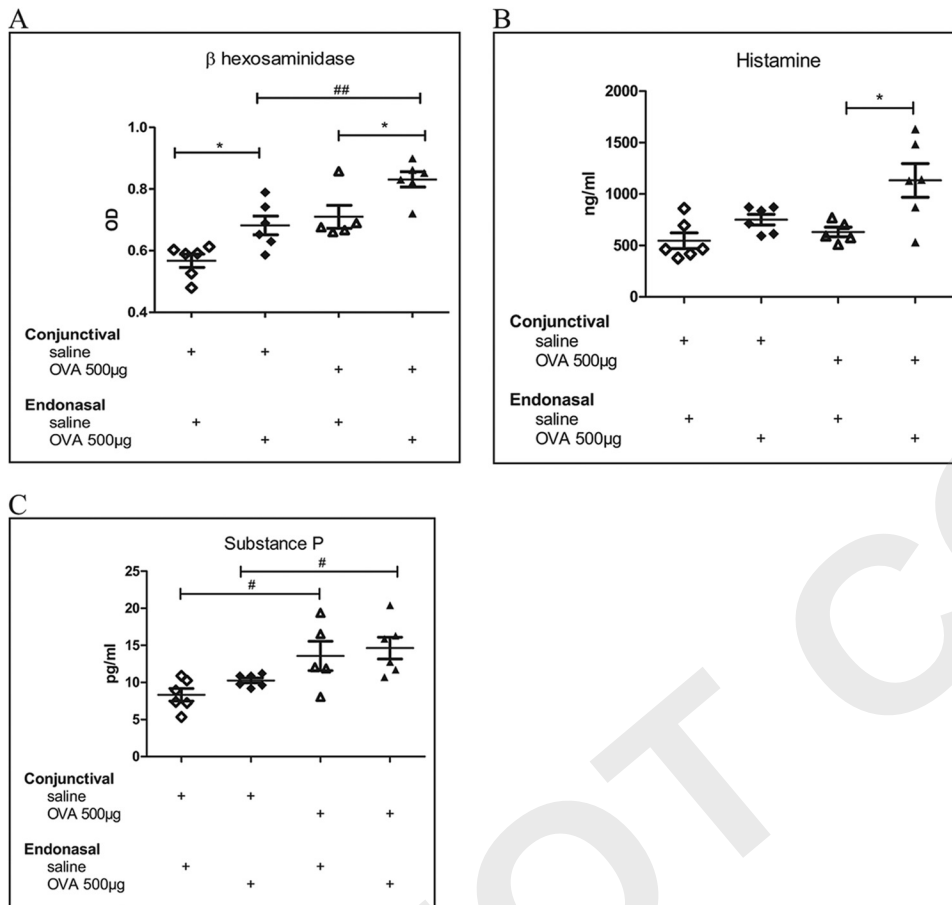


Figure 2. Levels of (A) β -hexosaminidase, (B) histamine, and (C) substance P in conjunctival (conj.) homogenates of mice 1 hour after challenge on day 17 (n = 6). Data are expressed as mean $\pm$ SEM; *p < 0.05; #p < 0.05; ##p < 0.01.

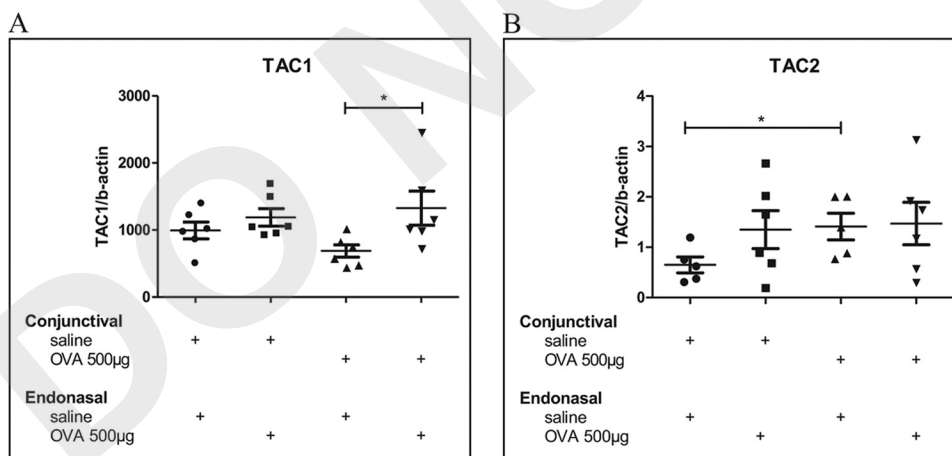


Figure 3. Levels of (A) protachykinin 1 (TAC1) and (B) TAC2 mRNA in trigeminal ganglia of mice 24 hours after last challenge on day 17 (n = 6). Data are expressed as mean $\pm$ SEM; *p < 0.05.

In line with the induction of conj. inflammation by nasal allergen challenges, e.n. challenge with OVA was associated with higher levels of EPO in conj. homogenates at 1 hour after the challenge than saline challenge (0.66 ± 0.09 nm versus 0.37 ± 0.03 nm; $p = 0.02$; data not shown).

Increased Expression of TAC1 and TAC2 in Trigeminal Ganglia after Nasal and/or conj. OVA Challenge

Higher mRNA expression of TAC1 was found in the trigeminal ganglia of mice e.n. and conj. challenged with OVA compared with

mice e.n. challenged with saline and conj. OVA (1326 ± 255 versus 687.5 ± 90.77 TAC1/ β -actin; $p = 0.04$; Fig. 3 A). Mice challenged with e.n. OVA showed no differences in TAC1 mRNA compared with mice challenged with e.n. saline after conj. saline challenge (1188 ± 130.2 versus 994.2 ± 125.1 TAC1/ β -actin; $p = 0.39$; Fig. 3 A).

Expression levels of TAC2 mRNA were significantly higher in mice challenged with conj. OVA compared with conj. saline after e.n. saline challenge (1.41 ± 0.26 versus 0.65 ± 0.16 TAC2/ β -actin; $p = 0.03$; Fig. 3 B). The e.n. challenge of OVA did not induce higher levels of TAC2 mRNA in mice that were conj. challenged with OVA (Fig. 3 B).

No differences in TAC1 and TAC2 expression levels in the trigeminal ganglia were found between all groups with similar

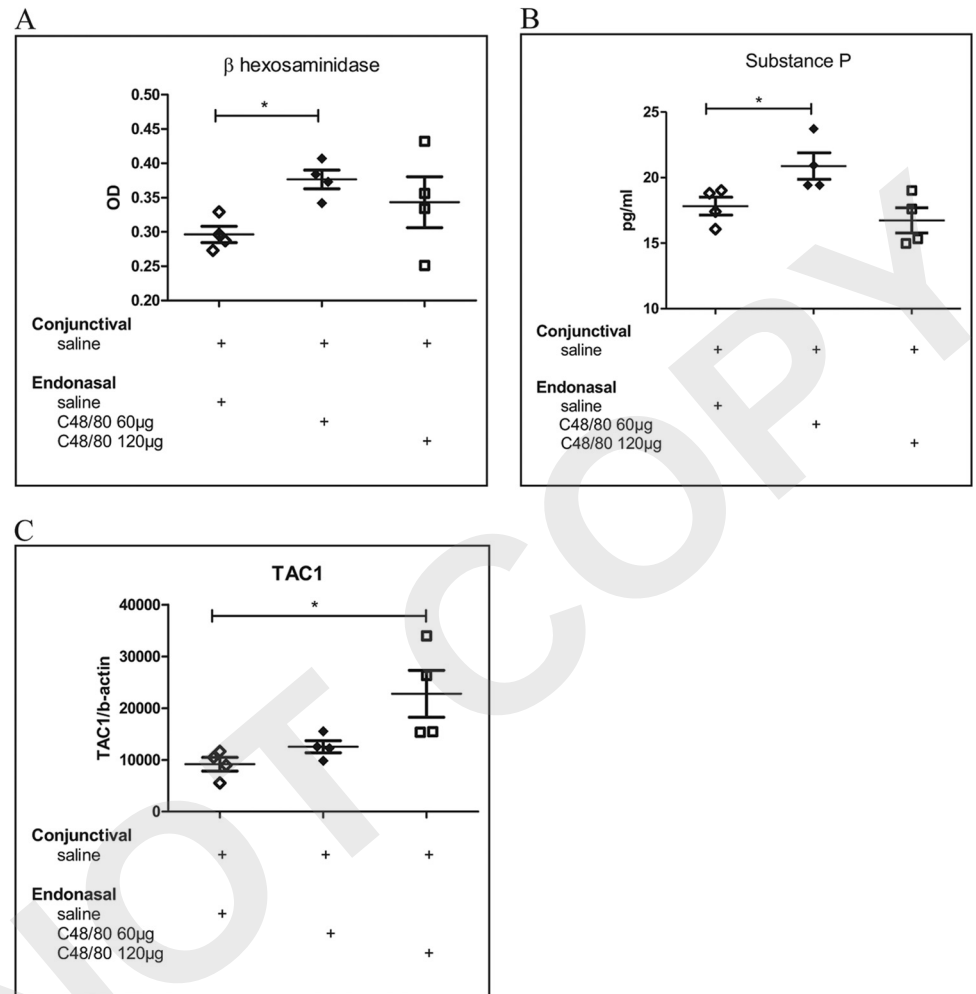


Figure 4. Levels of (A) β -hexosaminidase and (B) substance P in conjunctival (conj.) homogenates and (C) protachykinin 1 (TAC1) mRNA in trigeminal ganglia of mice endonasally (e.n.) challenged with saline, 60 μ g of C48/80, or 120 μ g of C48/80 together with saline in the conjunctiva ($n = 6$). Data are expressed as mean $\pm$ SEM; * $p < 0.05$.

conditions at 1 hour after the last allergen challenge (data not shown).

Nasal Mast Cell Stimulation by C48/80 Increased the conj. Release of β -Hexosaminidase, Substance P, and TAC1 Expression in Trigeminal Ganglia

Sensitized mice e.n. challenged with 60 μ g of C48/80 showed significantly higher levels of β -hexosaminidase compared with saline (0.37 ± 0.01 nm versus 0.29 ± 0.02 nm; $p = 0.02$, Fig. 4 A). In these mice, levels of substance P were also increased in conj. homogenates (20.88 ± 1.01 pg/mL versus 17.82 ± 0.68 pg/mL; $p = 0.02$; Fig. 4 B), whereas no differences in levels of histamine were found (data not shown). Of note, higher levels of TAC1 mRNA were observed in the trigeminal ganglia of mice after e.n. challenge with 120 μ g of C48/80 compared with saline (22785 ± 4537 versus 9179 ± 1318 TAC1/ β -actin; $p = 0.02$; Fig. 4 C). No significant differences in TAC2 mRNA were observed in the trigeminal ganglia of mice challenged with C48/80 or saline (data not shown).

Ketotifen Reduced the conj. Increase in Substance P and Increase in TAC1 Expression in Trigeminal Ganglia

Treatment with the mast cell stabilizer ketotifen reduced levels of substance P in conj. homogenates of mice e.n. challenged with OVA and conj. saline compared with vehicle (25.21 ± 1.24 pg/mL versus

28.88 ± 0.99 pg/mL; $p = 0.04$; Fig. 5 C). Also, TAC1 expression in trigeminal ganglia was significantly reduced in mice treated with ketotifen compared with vehicle (11128 ± 2218 versus 25723 ± 4271 TAC1/ β -actin; $p = 0.01$; Fig. 5 D). Levels of β -hexosaminidase and histamine in conj. homogenates did not significantly drop after ketotifen treatment compared with treatment with vehicle (Fig. 5, A and B). However, ketotifen treatment prevented the OVA-induced release of these mediators in conj. homogenates as seen in Fig. 2, A and B, where e.n. OVA challenge induced the release of these mediators in the conj. homogenates.

Nasal Capsaicin Abolished the OVA-Induced conj. Increase in Substance P, Histamine, and β -Hexosaminidase, as well as the Increase in Trigeminal TAC1 Expression

Mice received different doses of capsaicin e.n. at different time points. All used doses and time points were well tolerated without visible side effects as a consequence. Sensitized mice were pretreated e.n. with 1 mM of capsaicin on day 9 to destroy the sensory nerves in the nose.¹⁹ Indeed, these mice showed lower mRNA levels of PGP9.5 in nasal mucosa compared with mice treated with vehicle (21.39 ± 10.52 versus 157.2 ± 90.43 UCHL1/ β -actin; $p = 0.04$).

Significantly lower levels of β -hexosaminidase, histamine, and substance P were found in the conj. homogenates of mice pretreated with capsaicin compared with vehicle (Fig. 6, A–C). Moreover, these mice

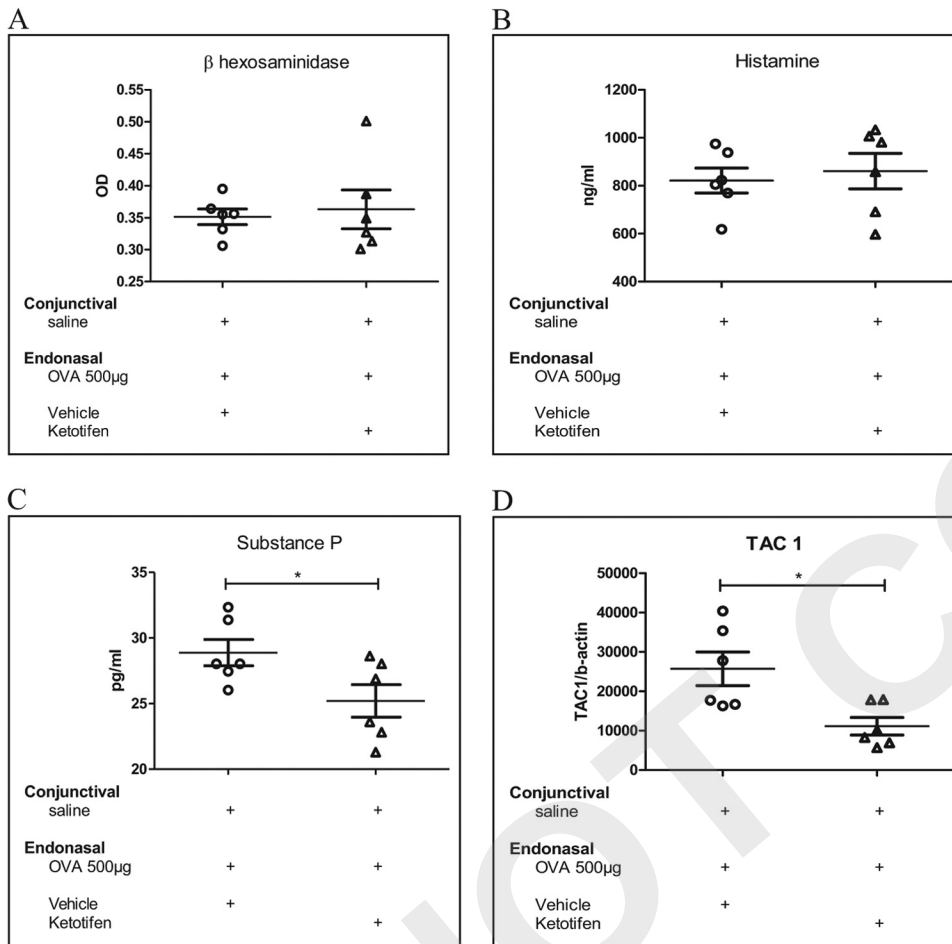


Figure 5. Levels of (A) β -hexosaminidase, (B) histamine, and (C) substance P in conjunctival (conj.) homogenates and levels of protachykinin 1 (TAC1) mRNA in (D) trigeminal ganglia in mice treated with ketotifen or vehicle and challenged with endonasal (e.n.) OVA and conj. saline ($n = 6$). Data are expressed as mean $\pm$ SEM; $*p < 0.05$.

did not show an OVA-induced increase in TAC1 mRNA in trigeminal ganglia after nasal OVA challenge (Fig. 6 D).

DISCUSSION

To explore the pathways involved in the naso-ocular interaction in allergic rhinoconjunctivitis, a mouse model of allergic rhinoconjunctivitis represents an attractive tool because it allows the dissection and study of trigeminal ganglia in relation to allergen deposition. Here, we show in a novel *in vivo* model of rhinoconjunctivitis that nasal allergen deposition in sensitized mice elicited the conj. release of histamine, β -hexosaminidase, and EPO, in parallel with elevated expression of TAC1 in trigeminal ganglia. Nasal mast cell activation was capable of inducing a similar conj. and trigeminal effect, with opposite effects on allergen-induced inflammation by treatment with ketotifen. Finally, destruction of nasal sensory nerves by capsaicin reduced the conj. and trigeminal response after nasal allergen challenge.

Here, we designed a novel model of allergic rhinoconjunctivitis allowing us to study the effects of nasal allergen stimulation on trigeminal and conj. parameters of disease. We found that nasal challenges with OVA resulted in higher levels β -hexosaminidase and histamine in conj. homogenates, in parallel with enhanced expression of TAC1 gene in the trigeminal ganglia. The naso-ocular interaction in AR is well described.^{2,3} The direct application of allergens on the nasal mucosa of allergic patients results in immediate conj. symptoms together with the presence of substance P and histamine in the tear fluid.² Triggering of the sensory nerve endings in the nasal airways by different stimuli leads to activation of the nerves and transmission of

axon potentials to the brain, resulting in defensive central reflexes such as sneezing by means of efferent signals. Besides this orthodromic pathway, sensory nerves can also be antidromically stimulated by action potentials originating at different terminals of the same neuron leading to the local release of neuropeptides such as substance P giving rise to vasodilatation, secretion, and increased vascular permeability by its local actions on the surrounding tissue. This phenomenon has been described as neurogenic inflammation. However, the mechanism behind this interaction remains unclear and it had already been suggested that histamine release by nasal mast cells activates sensory nerve endings in the nasal mucosa,^{2,12} ultimately leading to conj. release of substance P *via* stimulation of the trigeminal ganglia. Interestingly, we found increased expression of TAC1 in the trigeminal ganglia of mice after nasal OVA challenge. However, the rapid increase in substance P levels in tear fluid by allergen provocation most likely reflects the release of preformed neuromediators by the sensory nerve endings in the eye. The induction of mRNA of TAC1 represents *de novo* synthesis of neuromediators that may be released at later time points after activation and be responsible for a late-phase allergic response, as had been described in a guinea pig model of asthma.¹⁴ Indeed, nasal OVA increased conj. levels of β -hexosaminidase and substance P up to 24 hours after nasal challenge. Interestingly, mice showed a baseline expression of TAC1 and detectable levels of substance P in conjunctiva, suggesting a significant baseline neuroimmune homeostasis at the conj. surface by sensory nerve endings. Activation of the trigeminal ganglion will lead to the release of substance P among other neuromediators by sensory nerve endings on the ocular surface and conjunctiva. As was the case

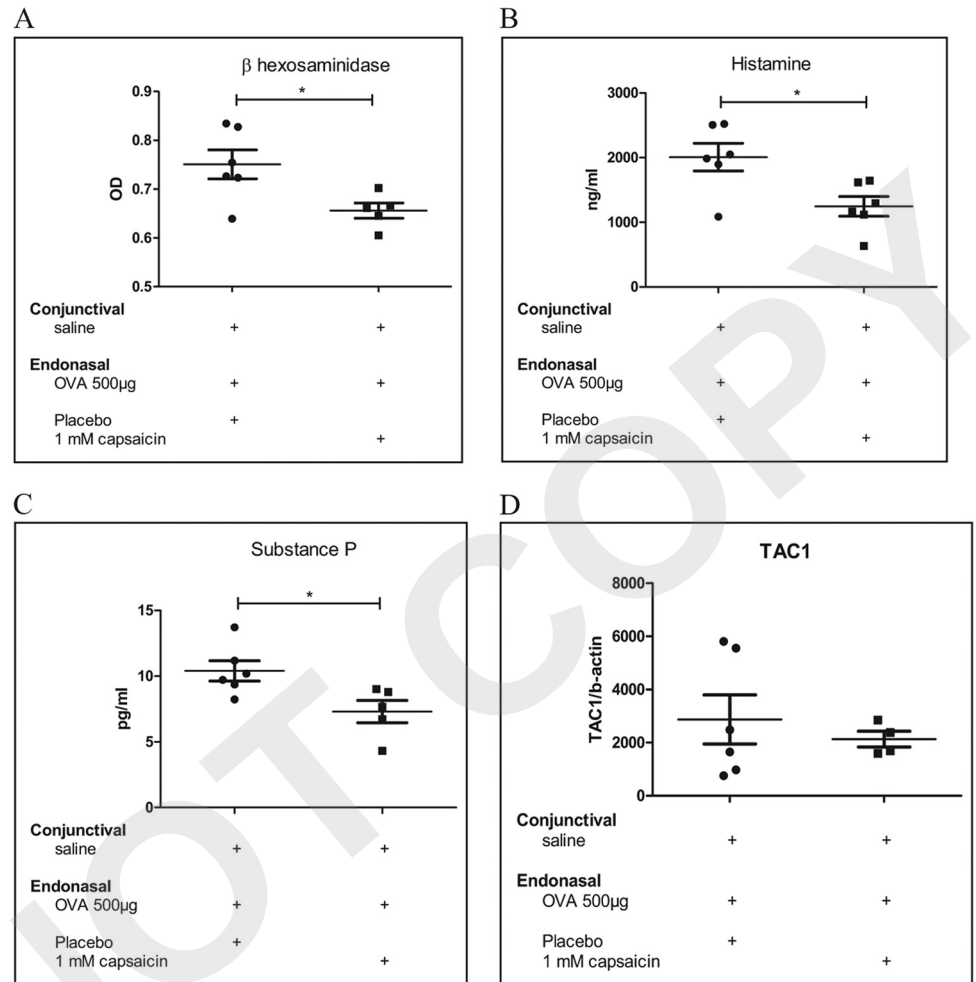


Figure 6. Levels of (A) β -hexosaminidase, (B) histamine, and (C) substance P in conjunctival (conj.) homogenates and (D) protachykinin 1 (TAC1) mRNA in trigeminal ganglia of mice treated with vehicle or 1 mM of capsaicin on day 9, 1 hour after endonasal (e.n.) OVA and conj. saline challenge ($n = 6$). Data are expressed as mean $\pm$ SEM; * $p < 0.05$.

in previously reported patient studies,^{2,3} only substance P showed detectable levels and other neuromediators such as neurokinin A and calcitonin gene-related peptide were below the detection limit in our model.

To explore the role of nasal mast cells as initiators of the naso-ocular interaction, we activated nasal mast cells by e.n. challenge with the mast cell activator C48/80. Application of C48/80 in the nose induced an expected mast cell degranulation, confirmed by increased levels β -hexosaminidase. However, in parallel, C48/80 led to elevated levels of substance P in the nose and higher expression levels of TAC1 in the trigeminal ganglia, suggesting an activation of the nasal sensory nerve endings by the released mast cell mediators. Therefore, these data indicate that mast cell activation and, hence, mediators released by mast cells play a role in the naso-ocular interaction, hereby confirming the hypothesis by Baroody *et al.*³ The fact that we do not observe a further increase in levels of conj. mediators after stimulation with the highest concentration of C48/80 may indicate that a maximum level of degranulation of mast cells is reached by the highest dose of C48/80.²⁰ Interestingly, the highest dose of C48/80 used, is, however, necessary to induce an increase in the levels of TAC1 mRNA in the trigeminal ganglia. The mast cell stabilizing agents with antihistamine characteristics such as ketotifen are commonly used in the treatment of asthma and allergic disorders. The prevention of degranulation reduces the amount of allergic symptoms in these patients. Ketotifen has been used in mouse models to highlight the role of mast cells in nonallergic airway hyperreactivity.²¹ Ketotifen has also been used in allergic mouse models as well as in

mouse models for colitis attenuating the disease.²² Here, ketotifen prevented the allergen-induced conj. mast cell degranulation and reduced conj. levels of substance P. In parallel with the hypothesis of involvement of the trigeminal ganglia, these mice showed reduced expression of TAC1 in the trigeminal ganglia.

Capsaicin is the active component of chili pepper and is known for its ability to activate or desensitize specific sensory fibers.^{12,23} Capsaicin binds the TRPV1 receptor, known as the pain receptor, on sensory nerves. Activation of TRPV1 by capsaicin results in a strong activation of the sensory nerves with the release of neuromediators and activation of the trigeminal ganglion in the brain.²⁴ Moreover, frequent dosing of capsaicin can cause a depletion of neuromediators from sensory nerves.²³ It has been described since 1991 that idiopathic rhinitis patients benefit from treatment with capsaicin²³ and it is shown that capsaicin exerts its therapeutic effects by ablating efferent nerves. Indeed, PGP9.5 stainings in human nasal mucosa after capsaicin treatment show a strong decrease in PGP9.5 on mRNA and protein level, confirming the hypothesis that capsaicin destructs the afferent nerves when applied intranasally. To evaluate the role of the afferent nerves in the mast cell-driven naso-ocular interaction, we destroyed the sensory nerve endings in the nose by capsaicin treatment before nasal allergen challenge. When capsaicin-treated and sensitized mice were e.n. challenged with OVA, a reduced secretion of substance P, β -hexosaminidase, and histamine was found in the conjunctiva. These data strongly support the important role of an intact sensory nervous system in the naso-ocular interaction in allergic rhinoconjunctivitis.

Levels of substance P, histamine, EPO, and β -hexosaminidase were elevated in conj. homogenates at 1 hour after the last challenge with e.n. OVA. However, 24 hours after the last challenge, only levels of β -hexosaminidase remained elevated and no differences were seen in substance P, EPO, and histamine levels. This is probably because of the immediate effect of the nasal allergen challenge on conj. inflammation that causes the early allergic response and because of the unstable character of substance P and histamine. However, 24 hours after the last challenge, increased expression levels of TAC1 were seen in the trigeminal ganglia of mice, indicating the *de novo* synthesis of neuromediators.

In conclusion, nasal allergen challenge of sensitized mice elicited a conj. mast cell degranulation together with trigeminal TAC1 expression. Both prevention of the allergen-driven mast cell degranulation by ketotifen and destruction of nasal sensory nerves by capsaicin reduced the conj. response after nasal allergen challenge. Therefore, these data reinforce the close interaction of mast cells and sensory nerves in the naso-ocular inflammation of allergic rhinoconjunctivitis.

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