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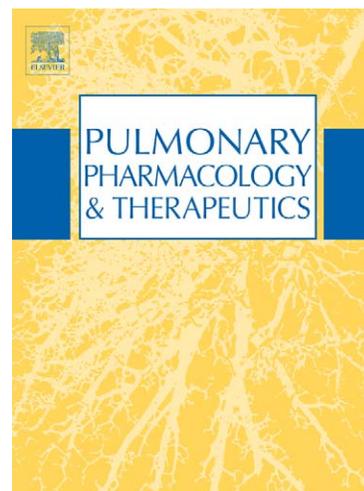
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Regular paper

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**ROLE OF THE TACHYKININ NK₁ RECEPTOR IN MEDIATING
CONTRACTION TO 5-HYDROXYTRYPTAMINE AND ANTIGEN IN
THE MOUSE TRACHEA**

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Abstract

Neuroimmune interactions are important in airway diseases such as asthma. We evaluated the role of the tachykinin NK₁ receptor in the contractile response of isolated trachea from tachykinin NK₁ receptor wild-type and knockout mice, to the antigen ovalbumin and the contractile agonist serotonin (5-hydroxytryptamine). 1% ovalbumin induced contractions of tracheas obtained from ovalbumin-immunized and exposed mice. The tracheas from wild-type animals showed larger contractions compared to the knockout mice. Tracheas from sensitised and ovalbumin-exposed animals released 5-hydroxytryptamine upon addition of ovalbumin. Higher levels of 5-hydroxytryptamine were released from tracheas of wild-type animals. Tracheas of non-sensitised animals did not release 5-hydroxytryptamine upon ovalbumin challenge. Responses to ovalbumin were abrogated by methysergide, a broad 5-hydroxytryptamine receptor antagonist. Exogenous 5-hydroxytryptamine contracted tracheas but wild-type tracheas responded significantly more. Atropine and tetrodotoxin reduced 5-hydroxytryptamine-induced contractions of the wild-type tracheas, while they did not affect 5-hydroxytryptamine-induced contractions of knockout tracheas. 5-hydroxytryptamine induced contractions from atropine- or tetrodotoxin-treated wild-type tracheas did not differ significantly from the contractions of the knockout tracheas. Single tachykinin NK₁ receptor antagonists SR140333 and RP67580 had no effect on 5-hydroxytryptamine-induced contractions. In conclusion, the 5-hydroxytryptamine-induced tracheal contraction includes a cholinergic mechanism that requires the presence of the tachykinin NK₁ receptor.

Keywords

Substance P, knockout mice, airway contraction, tachykinins, 5-hydroxytryptamine, asthma

1. Introduction

Substance P is a member of the tachykinin peptide family and is localized in the sensory afferent nerves and inflammatory cells of the airways. Structural elements, like epithelium may be an additional source. Tachykinins may be released by a variety of stimuli (e.g. cigarette smoke, ozone) and they generate a range of effects that are potentially relevant in the pathogenesis of asthma [1]. Their effects are mediated through specific receptors, the tachykinin NK₁, NK₂ and NK₃ receptors. In the airways, tachykinin NK₁ receptors, the high affinity receptors for substance P, are mainly involved in proinflammatory effects while NK₂ receptors, preferential receptors for neurokinin A, are considered to be important in smooth muscle contraction.

Airways of atopic asthmatics respond to inhalation of allergen with an immediate bronchoconstriction, the early asthmatic response. This bronchoconstriction is induced by IgE-mediated release of mast cell mediators like prostaglandins, cysteinyl-leukotrienes and others [2]. The importance of mast cells in the antigen-induced bronchoconstriction has also been demonstrated in various animal models, including mice [3;4]. In the airways, a close anatomical relationship exists between mast cell and neurons and these neurons have been shown to be immunoreactive for substance P (reviewed in [5]). A direct interaction between these two cell types has been suggested [6]. Functional evidence exists for the presence of tachykinin NK₁ receptors on mast cells [7-9]. Substance P is able to induce degranulation of human and rat mast cells, which can lead to bronchoconstriction [10;11]. Low concentrations of substance P can prime or sensitise mast cells to other stimuli [12]. The interaction may be bi-directional. In vitro, neurites are activated in response to mast cell activation [6] and many studies have shown that mast cell derived products like histamine and 5-HT modulate sensory neurotransmission [13-15]. Therefore, a role for the tachykinin NK₁ receptor in mast cell mediated bronchoconstriction could be suspected. However, a clear involvement has not been demonstrated yet.

Exogenous 5-HT is also known to cause bronchoconstriction in rats and mice. However, the observed bronchoconstriction in rodents is strain dependent [16]. 5-HT can have a direct contractile effect by the interaction with 5-HT₂ receptors on airway smooth muscle and an indirect effect by stimulation of cholinergic nerves and the subsequent release of acetylcholine [17-19]. Recently, it was shown in mice that 5-HT was able to release acetylcholine from the epithelium as well [20]. It is important to know by which exact mechanism 5-HT contract the airways as rodents are often used in disease models for asthma. As 5-HT modulates sensory neurotransmission, the tachykinin NK₁ receptor might be

involved in the response to exogenous 5-HT too. Furthermore, in the central nervous system it was shown that the absence of the tachykinin NK₁ receptor resulted in the functional desensitisation / downregulation of 5-HT receptors [21].

The aim of our study was to examine a possible involvement of the tachykinin NK₁ receptor in the tracheal contractile response to antigen and to exogenous 5-HT. Therefore, we evaluated 5-HT- and antigen-induced tracheal smooth muscle contraction from mice lacking the tachykinin NK₁ receptor and compared this response to that of the wild type counterparts. Our results demonstrate an involvement of the tachykinin NK₁ receptor in 5-HT-induced smooth muscle contraction and in antigen-induced smooth muscle contraction by interaction with cholinergic mechanisms.

2. Materials and methods

2.1. Mice:

Tachykinin NK₁ receptor wild type (WT) and knockout (KO) mice were derived as described from the mating of heterozygous tachykinin NK₁ receptor^{+/-} mice [22]. The targeting construct was derived from a mouse 129/sv strain genomic library and targeted clones were injected into C57Bl/6 blastocysts. Chimaeric males were mated with C57Bl/6 females. The mice were bred from successive generations of sibling KO and WT mice and can be thought of as representing a recombinant inbred strain. The respective tachykinin NK₁ receptor KO and WT breeding pairs were provided by the lab of S. Hunt (Cambridge, UK). The animals were bred locally and maintained under germ free conditions in a conventional animal house in the animal research facilities of the Department of Respiratory Diseases of the University Hospital, Ghent and received food and water *ad libitum*. The used protocols comply with the European Community guidelines and were approved by the local ethics committee.

2.2. Immunization and exposure of mice:

On day 0, test mice (female, 8-10 weeks) were actively immunized with ovalbumin (Grade III; Sigma chemical Co.) by intraperitoneal injection of 10 µg ovalbumin, adsorbed to 1 mg Al(OH)₃. Control mice received only adjuvant. From day 14 to 21, the mice were exposed daily to phosphate buffered saline (PBS) or ovalbumin aerosols (1%) for 30 min in a Plexiglas exposure chamber (30x20x15 cm). The aerosol was generated by an ultrasonic nebulizer (Ultraschallvernebler Sirius Nova, Heyer Medizintechnologie, Bad Ems, Germany). According to the specifications of the manufacturer, the output of the nebulizer was 3 ml/min and the mean particle size of the aerosol was 3.2 µm. 24 hours after the last exposure, mice were sacrificed and tracheas were excised.

2.3. General procedure:

All experiments were performed with female mice of 8 to 10 weeks of age. The mice were killed by an intraperitoneal injection of pentobarbital (Nembutal[®], 100 mg/kg). The trachea was gently excised and the adhering connective tissue was carefully dissected and removed under microscopy guidance. The whole tracheas were mounted in a 2 ml organ bath containing Krebs solution (composition in mM: NaCl 118, KCl 4.6, CaCl₂ 2.5, MgSO₄ 1.15, NaHCO₃ 24.9, KH₂PO₄ 1.15 and glucose 5.5), which was maintained at 37°C and bubbled with carbogen (5% CO₂, 95 % O₂). The optimal resting tension on the trachea of this mouse strain was 0.7 g, as determined by length-tension experiments. The tracheas were allowed to stabilize for 15 minutes at this resting tension. After this stabilization period, increasing concentrations of carbachol were added to the organ bath in a non-cumulative way (0.33, 1.00 and 3.3 μM). The contact time for each concentration was 15 minutes. Between the administrations, an interval of 10 min was inserted, while the bathing medium was changed. This was followed by a resting period of 30 minutes while the bathing medium being changed every 10 minutes. The contractions were measured isometrically with Grass FT03 transducers (Grass Instruments Co., Quincy, MA, USA) and recorded on a Graphtec Linearecorder type WR3701 (Graphtec corp. Tokyo, Japan). Contractions were expressed as percent of the contraction that was observed using a maximal concentration of carbachol, as tracheas of both tachykinin NK₁ receptor WT as KO animals were shown previously to respond similarly to carbachol [23].

2.4. Effect of ovalbumin on the tracheal ring tension:

The tracheas were exposed to ovalbumin by adding 1% ovalbumin in Krebs solution into the organ bath. The effect of methysergide (10⁻⁷ M) was evaluated by adding this 5-HT antagonist to the bathing medium 30 minutes before the addition of ovalbumin.

2.5. In vitro release of 5-HT:

At the moment of maximal contraction induced by ovalbumin, 200μl was taken from the organ bath to measure 5-HT. 5-HT was measured by the use of an enzyme-immunoassay. The assay is based on the competition of acylated 5-HT and the acylated enzyme-conjugate 5-HT-acetylcholinesterase for an antibody against acylserotonin (Immunotech, Marseilles, France). The sensitivity of this assay is 0.5nM.

2.6. Effects of histamine and 5-HT on the tracheal ring tension:

Tracheas were excised from control, non-immunized, non-exposed mice. Increasing concentrations of histamine (10⁻⁸ to 10⁻⁴ M) were added to the organ bath in a non-cumulative way (in steps of 0.5 log [concentration]), whereby the contact time for each concentration was

at least 4 minutes. Concentration-response curves to 5-HT were constructed after the stabilization period with carbachol and the subsequent resting period, by adding increasing concentrations to the organ bath in a cumulative way (10^{-8} M to 10^{-4} M). The contact time for each concentration was at least 4 min. Due to the occurrence of tachyphylaxis, only one concentration-response curve of 5-HT could be obtained. The effect of methysergide (10^{-7} M) was evaluated by adding the 5-HT antagonist to the bathing medium 30 minutes before the construction of the concentration-response curve to 5-HT.

2.7. Modulation of the 5-HT-induced contractions by atropine, tetrodotoxin, SR140333 and RP67580:

A concentration-response curve to 5-HT was performed as previously described in the presence and absence of atropine (10^{-6} M), tetrodotoxin (TTX) (10^{-6} M), SR140333 (10^{-7} M) and RP67580 (10^{-7} M). The substances were added to the bathing medium, starting from the resting period and during the construction of the concentration-response curve. Non-immunized, non-exposed animals were used.

2.8. Materials:

Most drugs and chemicals were purchased from Sigma Chemical Co. (Poole, UK). Nembutal was from Sanofi (Libourne, France). PBS was from Gibco (Invitrogen, Merelbeke, Belgium). The tachykinin NK₁ receptor antagonist SR140333 was a gift from X. Emonds-Alt (Sanofi Recherche, Montpellier, France). RP67580 was obtained from Tocris Cookson Ltd. (Avonmouth, UK). Stock solutions of these antagonists were prepared with ethanol p.a. (Merck, Brussels, Belgium) and further diluted in Krebs solution as appropriate. TTX was dissolved in ultrapure water. Other stock solutions were prepared with Krebs solution. All dilutions of drugs were prepared on the day of the experiments.

2.9. Statistical analysis:

The results are reported as mean \pm standard error of the mean (SEM). Concentration-response curves in the absence and presence of antagonists, TTX and atropine (multiple comparisons) were analysed with the General Linear Model Univariate procedure, providing regression analysis and analysis of variance for one dependent variable by two factors (the groups and the concentrations). Post-hoc analysis (Scheffé) for groups was performed when significance was reached. The differences in ovalbumin-induced contractions between groups were evaluated using Kruskal Wallis H test and Mann Whitney U for comparing individual groups. Significance levels were adapted with Bonferroni's conservative correction for repetitive measurements. Differences were regarded as significant when $p < 0.05$. The

statistical analyses were accomplished with SPSS for Windows 11.0 software (SPSS Inc., Chicago, Illinois).

3. Results

3.1. Contractile response of tracheas to ovalbumin

Addition of 1% ovalbumin into the organ bath induced a marked contraction in both the immunized and exposed tachykinin NK₁ receptor WT and KO animals. This reaction was significantly larger in the WT animals (n = 6) (p<0.05) (table 1). Tracheas from sham-immunized and PBS exposed mice did not respond to the antigen. Ovalbumin induced the release of 5-HT in the organ bath but no differences were observed between the tracheas from tachykinin NK₁ receptor WT and KO animals (WT: 2.3 nM ± 0.9; KO: 4.3 nM ± 1.5, p>0.05; n = 4; non-sensitized animals: below detection limit). Pretreatment with the 5-HT antagonist methysergide abolished the response to ovalbumin completely in both WT and KO animals (p<0.05 vs. solvent treated counterparts, n = 4) (table 1).

3.2. Contractile response of tracheas to 5-HT and histamine

The tracheas of the non-immunized, non-exposed tachykinin NK₁ receptor WT and the KO animals did not contract after histamine addition (n = 5, results not shown). 5-HT induced contractions in a concentration-dependent way. A bell-shaped concentration-response curve was observed (figure 1). The tracheas of the WT mice responded significantly more to the increasing concentrations of 5-HT in comparison with the tracheas of the tachykinin NK₁ receptor KO mice (n = 8) (p<0.05) (figure 1). All responses to 5-HT were abolished in the presence of methysergide (results not shown).

3.3. Modulation of the 5-HT-induced contractions by atropine

Pre-treatment of the tracheas of non-immunized, non-exposed mice with 10⁻⁶ M atropine reduced the 5-HT-induced contractions in the tachykinin NK₁ receptor WT (p<0.05 vs. solvent-treated WT tracheas) (figure 2) (n = 5). At a concentration of 10⁻⁶ M, atropine had no effect on the 5-HT-induced contractions of the tracheas from KO mice (n = 5) (p>0.05 atropine- vs. solvent-treated KO tracheas) (figure 2). 5-HT-induced contractions of atropine-treated WT tracheas did not differ significantly from the tracheal contractions of the KO animals (p>0.05 vs. solvent- and atropine-treated KO tracheas) (figure 2).

3.4. Modulation of the 5-HT-induced contractions by TTX

To evaluate possible neural effects, the response to 5-HT was studied in the presence of the neurotoxin TTX (10⁻⁶ M). Tracheas obtained from non-immunized, non-exposed tachykinin NK₁ receptor WT mice responded significantly less to 5-HT in the presence of TTX (p<0.05

vs. solvent treated tracheas, $n = 5$). Concentration-response curves of 5-HT on tracheas from KO animals were not influenced by TTX ($p > 0.05$ TTX- vs. solvent-treated KO tracheas). The observed responses of the WT tracheas were not significantly different from responses obtained in tracheas from tachykinin NK₁ receptor KO mice ($p > 0.05$ vs. solvent- and TTX-treated KO tracheas) (figure 3).

3.5. Modulation of the 5-HT-induced contractions by the tachykinin NK₁ receptor antagonists SR140333 and RP67580

Pre-treatment of the WT tracheas with the tachykinin NK₁ receptor antagonists RP67580 (10^{-7} M) and SR140333 (10^{-7} M) did not influence the 5-HT-induced contractions. No significant differences were observed between concentration-response curves from solvent-treated WT tracheas and antagonist-treated WT tracheas ($n = 6$, figure 4A and B respectively).

4. Discussion

Tracheal tissue from mice sensitised and exposed to ovalbumin showed an immediate contractile response to the antigen *in vitro*. However, tracheas from mice lacking the tachykinin NK₁ receptor responded significantly less compared to their WT counterparts. Exogenous 5-HT induced also contraction of the tracheal smooth muscle of non-immunized, non-exposed animals. Again, the tracheas from the tachykinin NK₁ receptor KO animals responded significantly less. Responses to antigen and 5-HT were abolished in the presence of methysergide. Atropine and TTX did not influence responses to 5-HT in tachykinin NK₁ receptor KO mice but lowered 5-HT-induced contractions in WT tracheas. No effect of tachykinin NK₁ receptor antagonists SR140333 and RP67580 vs. 5-HT could be demonstrated in WT tracheas.

The observed contractile response to antigen is due to activation of mast cells via antigen cross-linking of IgE antibodies bound on the high affinity IgE receptor FcεRI [24]. It is important to note that no differences in serum ovalbumin-specific IgE levels between the tachykinin NK₁ receptor WT and KO mice can be detected [25]. Upon activation, mast cells degranulate, thereby releasing a variety of mediators like histamine and 5-HT, which in their turn induce contraction [4;26]. Indeed, after stimulation with the antigen, the presence of 5-HT in the organ bath was demonstrated. Furthermore, methysergide, a 5-HT₁ and 5-HT₂ combined receptor antagonist, abolished the contractile response to ovalbumin. These findings are in accordance with the findings of Nagase and colleagues. They reported a specific role for 5-HT₂ receptors in antigen-induced airway smooth muscle constriction [27]. Also Eum and colleagues demonstrated the importance of 5-HT in antigen-induced

contractions [19]. As equal amounts of 5-HT were released from tracheas of tachykinin NK₁ receptor WT and KO animals, it is suggested that the tachykinin NK₁ receptor was not involved in the triggering of the mast cell by the antigen and the subsequent release of its mediators. Although histamine can be released from murine mast cells, exogenous histamine did not elicit contractions in this model. This is in agreement with previous findings obtained in rat [28;29]. Exogenous 5-HT induced tracheal contractions but responses were smaller in tachykinin NK₁ receptor KO animals. Responses to 5-HT were abolished by the presence of methysergide demonstrating a receptor-mediated event. The contractile effect of 5-HT has previously been demonstrated in rats [17] and in mice but this depended on the inbred strain of mice used [16;18]. Often, a cholinergic component was demonstrated. Furthermore, 5-HT has a facilitatory effect on cholinergic neurotransmission in human [30], mouse [18], rat [31] and guinea pig [32] airways. In our model, a cholinergic component is also demonstrated. Atropine reduced the contractile response to 5-HT in the tachykinin NK₁ receptor WT tracheas, suggesting the 5-HT-induced release of acetylcholine. We hypothesized that the acetylcholine arose from cholinergic nerve endings. The neural character of this phenomenon was confirmed by the fact that TTX, a neurotoxin, also reduced the 5-HT-induced contractions of tracheas from the tachykinin NK₁ receptor WT mice. However, neither atropine nor TTX influenced the 5-HT-induced tracheal contractions of tachykinin NK₁ receptor KO mice. This suggests that the tachykinin NK₁ receptor is pivotal for the 5-HT-induced release of acetylcholine. A different sensitivity of the tracheal smooth muscle to 5-HT between the tachykinin NK₁ receptor WT and KO mice is not very likely since there were no longer differences between the contractile responses of both groups to 5-HT in the presence of atropine or TTX. Furthermore, we previously showed that the tracheal smooth muscle preparation of the WT and KO mice respond similar to the cholinergic agonist carbachol [23]. A generalized artifact of cholinergic nerve activity seems unlikely too, since electrical field stimulation of the tracheas from NK₁ receptor knockout animals still generated acetylcholine release and acetylcholine-mediated contraction although this release and contraction were diminished compared to the response in the WT counterparts [23].

The difference in the OA-induced contractions between the tachykinin NK₁ receptor WT and KO animals seems larger than the observed difference between the concentration-response curves to 5-HT. It is important to notice that the response to OA was evaluated with a single concentration of the OA while the effects of exogenous 5-HT were evaluated with a concentration-response curve. This may lead to a misinterpretation of the results. However, it seems plausible that due to the close relationship between mast cells and nerves, the effect of

the endogenously released 5-HT is more extensively directed towards the proximal cholinergic nerves while in the response to exogenous 5-HT, the muscular component becomes more important.

There are several possible explanations for the involvement of the tachykinin NK₁ receptor in the 5-HT induced release of acetylcholine from cholinergic nerve endings. Functional evidence exists for the presence of 5-HT receptors on sensory nerve endings. Activation of these receptors with their ligand 5-HT may result in the release of tachykinins [33;34]. The released substance P may in its turn facilitate or permit the release of acetylcholine [23]. Such cascade has previously been suggested in guinea pig ileum [35]. However, we dismissed this hypothesis by the use of specific tachykinin NK₁ receptor antagonists. SR140333, a specific NK₁ receptor antagonist, did not influence the 5-HT induced contractions of tracheas from tachykinin NK₁ receptor WT animals. The used concentration of SR140333 is sufficient to antagonize the tachykinin NK₁ receptor sufficiently as was previously demonstrated in our model [23]. Higher concentrations of the tachykinin NK₁ receptor antagonist were no longer specific as tracheas from tachykinin NK₁ receptor KO animals were also influenced (results not shown). Furthermore, another specific tachykinin NK₁ receptor antagonist RP67580 also failed to influence the 5-HT induced contractions of tracheas from the tachykinin NK₁ receptor WT mice. Again, the used concentration should be sufficient as the K_i value for this antagonist is 2.9 nM for rat tachykinin NK₁ receptors. These antagonist studies suggest that the tachykinin NK₁ receptor is not involved in the immediate contractile response to 5-HT. Similar findings were reported in guinea pigs [32]. However, long-term blockade of the tachykinin NK₁ receptor may induce developmental compensations that lead to the observed difference in the cholinergic aspect of the 5-HT induced tracheal contractions. In the central nervous system, the lack of the tachykinin NK₁ receptor resulted in the functional desensitisation / downregulation of the presynaptic 5-HT_{1A} receptor [21;36]. Our results suggest a similar downregulation of 5-HT receptors on peripheral nerve endings in our mouse model.

To conclude, our data confirm previous findings that the immediate response to allergen in mouse trachea in vitro is mediated through the release of 5-HT from mast cells. The released 5-HT mediates contraction through activation of receptors on smooth muscle and through activation of presynaptic receptors on cholinergic nerve endings. We found that this cholinergic mechanism is impaired in mice lacking the tachykinin NK₁ receptor, probably through downregulation of the peripheral presynaptic 5-HT receptors on cholinergic nerve endings (figure 5). It is tempting to speculate that long-term addition of tachykinin NK₁

receptor antagonists may render cholinergic nerve endings less sensitive to mast cell mediators which may have important implications in mast cell related disorders such as asthma.

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Legends

Figure 1

In vitro responses to increasing concentrations of 5-HT of tracheas from non-immunized, non-exposed tachykinin NK₁ receptor knockout mice (closed circles, hatched line) versus wild-type mice (closed squares, closed line) (n=8). Data are presented as mean ± SEM. Tracheas from wild-type mice responded significantly more to 5-HT compared to tracheas from knockout mice.

Figure 2

In vitro responses to increasing concentrations of 5-HT of tracheas from non-immunized, non-exposed tachykinin NK₁ receptor knockout mice (circles, hatched line) and wild-type mice (squares, closed line) in the presence (open symbols) and absence (closed symbols) of atropine (10⁻⁶ M) (n=5). Data are presented as mean ± SEM. Atropine decreased the responses of wild-type tracheas to 5-HT down to a level comparable with the responses from the knockout tracheas (p<0.05 wild-type solvent vs. atropine). Responses of knockout tracheas to 5-HT were not influenced by atropine.

Figure 3

In vitro responses to 5-HT from tracheas of non-immunized, non-exposed tachykinin NK₁ receptor knockout (circles, hatched line) and wild-type mice (squares, closed line) in the presence (open symbols) and absence (closed symbols) of TTX (10⁻⁶ M) (n=5). Data are presented as mean ± SEM. TTX-treated wild-type tracheas responded significantly less to 5-HT compared to solvent treated counterparts (p<0.05). Responses from TTX-treated wild-type tracheas were not significantly different from responses of knockout tracheas. Responses of knockout tracheas to 5-HT were not influenced by TTX.

Figure 4

In vitro responses to 5-HT from tracheas from non-immunized, non-exposed tachykinin NK₁ receptor WT mice in the presence (open squares) and absence (closed squares) of the tachykinin NK₁ receptor antagonists RP67580 (10⁻⁷ M, n=6) (A) and SR140333 (10⁻⁷ M, n=6) (B). Data are presented as mean ± SEM. Responses were not influenced by these antagonists.

Figure 5

Proposed mechanism to explain the difference in response to albumin and 5-HT between NK₁R knockout and WT trachea: Addition of the allergen ovalbumin to sensitized mouse trachea in vitro causes the release of 5-HT from mast cells through activation of the high affinity IgE receptor FcεRI. The released 5-HT mediates contraction through activation of receptors on smooth muscle and through activation of presynaptic receptors on cholinergic nerve endings, which induce the release of acetylcholine (ACh). This cholinergic mechanism is impaired in mice lacking the tachykinin NK₁ receptor, probably through desensitisation / downregulation of the peripheral presynaptic 5-HT receptors on cholinergic nerve endings.

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TABLE 1: OVALBUMIN-INDUCED CONTRACTIONS OF MOUSE TRACHEA

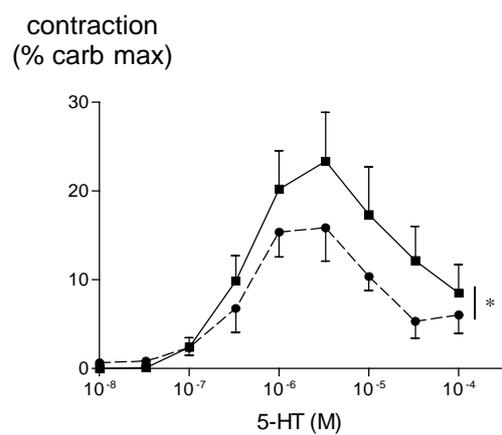
| Treatment | WT (% carb. max.) | | KO (% carb. max.) | |
|-------------|-------------------|--------------|-------------------|--------------|
| | solvent | methysergide | solvent | methysergide |
| PBS-exposed | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.4 ± 0.4 | 0.0 ± 0.0 |
| OA-exposed | 11.6 ± 2.9* ‡ | 0.2 ± 0.3† | 4.2 ± 1.5* | 0.6 ± 0.4† |

Definition of abbreviations: WT = tachykinin NK₁ receptor wild type, KO = tachykinin NK₁ receptor knockout, PBS = phosphate buffered saline, OA = ovalbumin. n = at least 4.

Data as mean ± SEM. *p < 0.05 vs PBS; †p < 0.05 vs solvent, ‡p < 0.05 vs KO

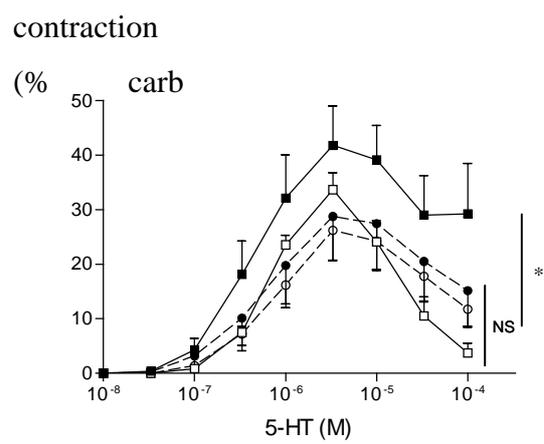
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FIGURE 1



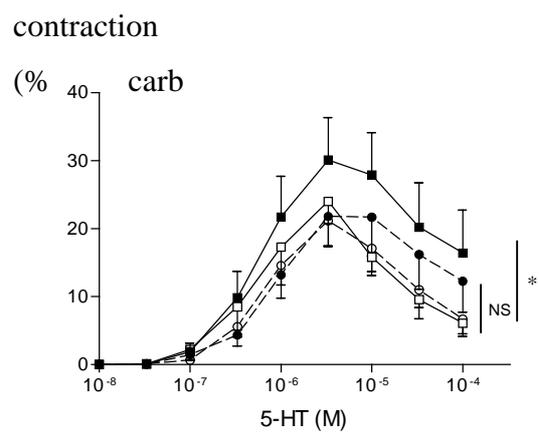
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FIGURE 2



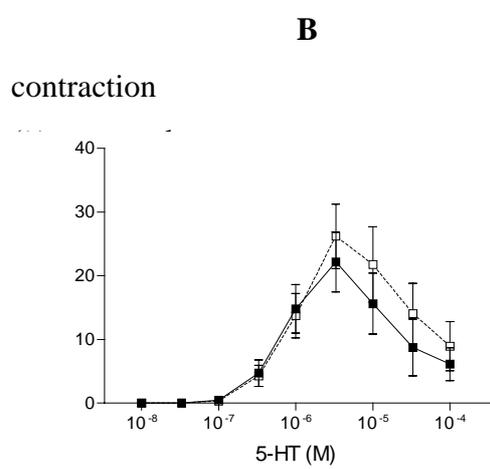
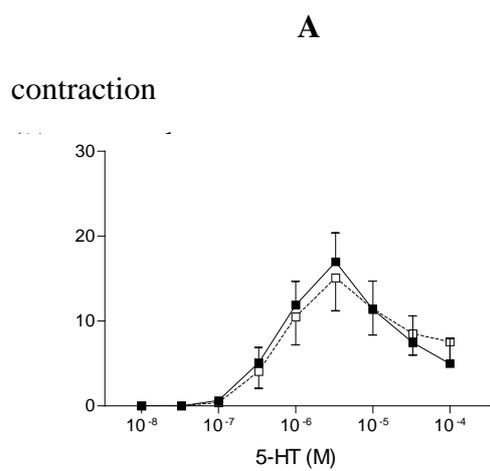
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FIGURE 3



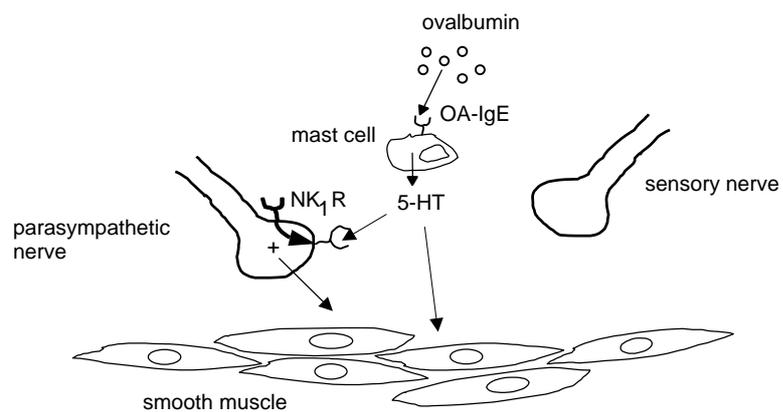
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FIGURE 4



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FIGURE 5



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